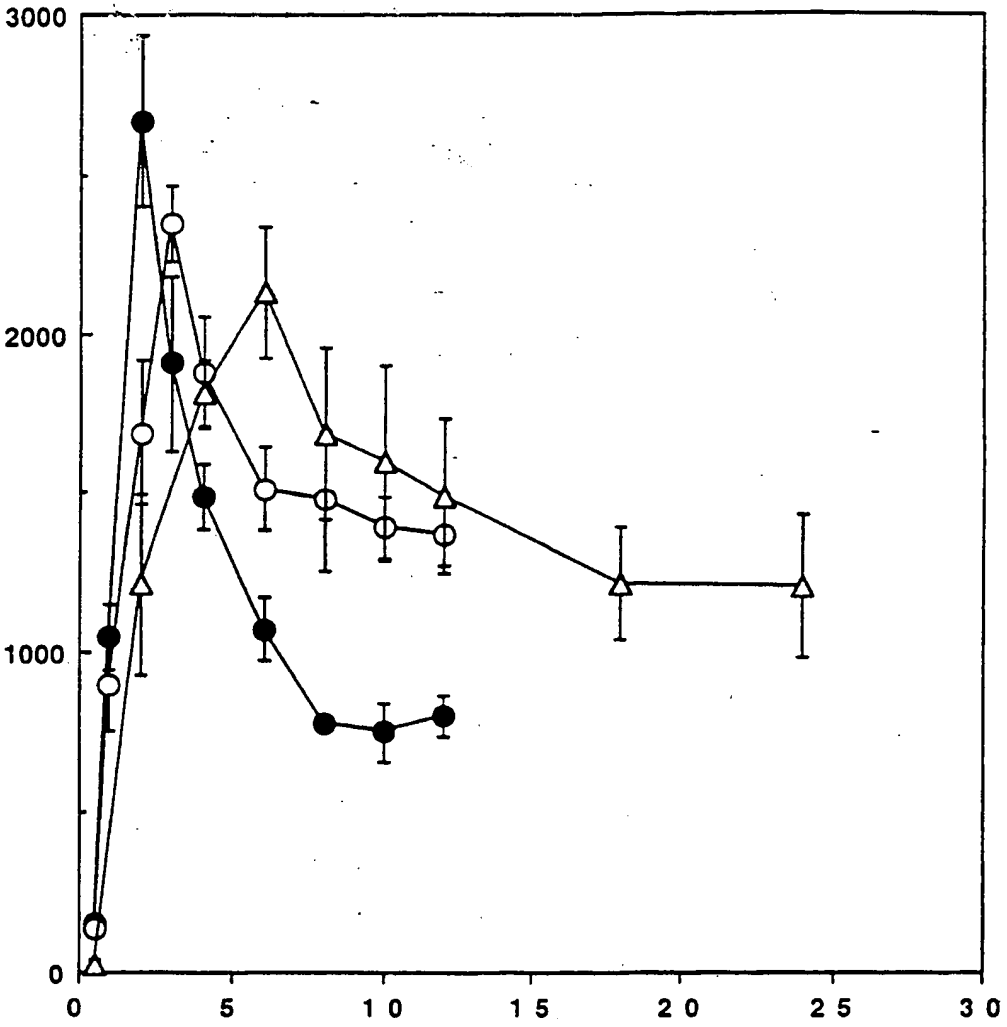
FIGURE 1

EFFECT OF TEMPERATURE ON THE SPECIFIC BINDING OF 5 nM ^{3}H-E_2 TO MTW9/PL2 CELLS

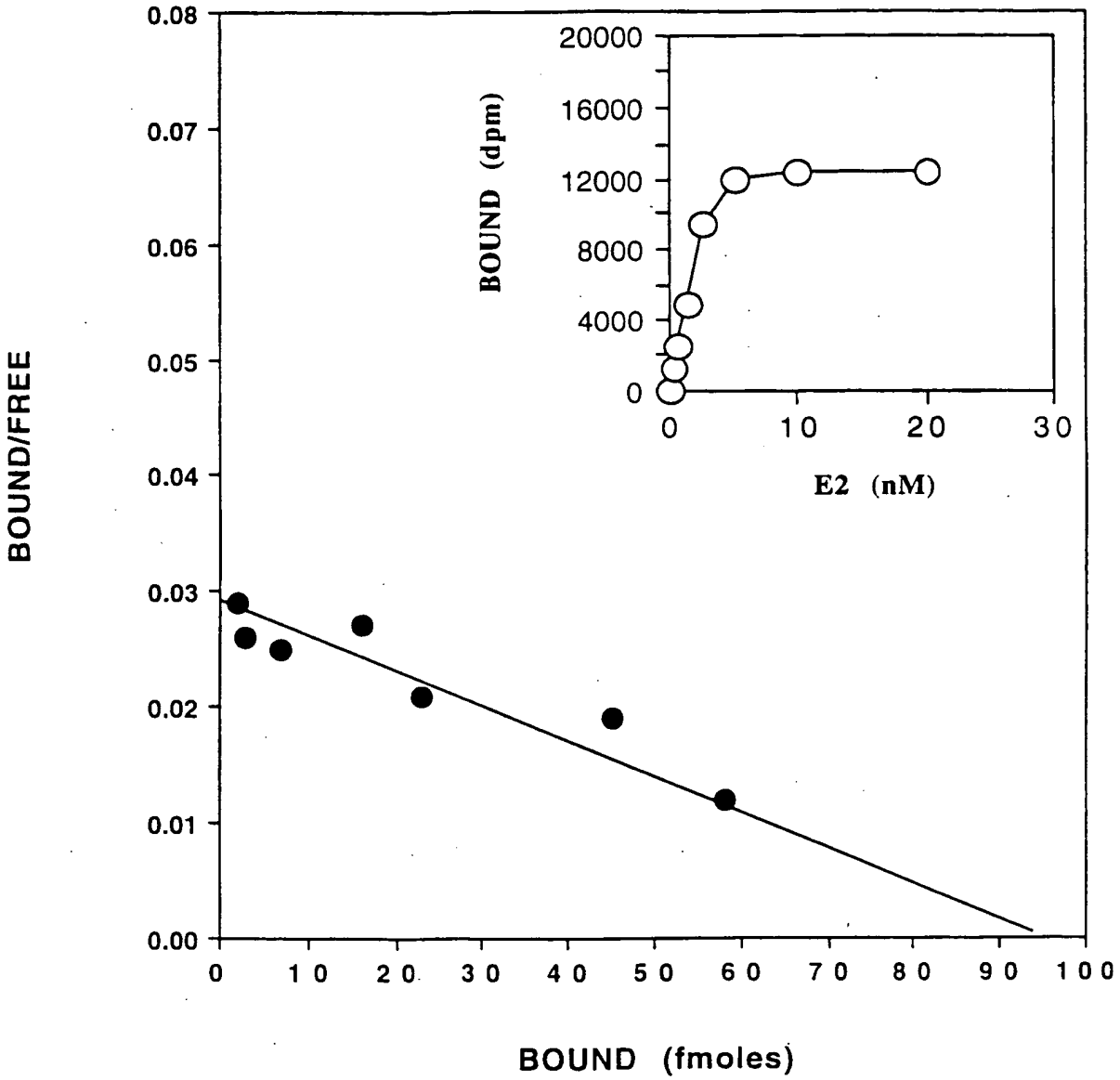


The kinetics are shown (± SD of triplicates) at 37° C (closed circles), 23° C (open circles), and at 4° C (open triangles).

Specific binding was determined in phenol red-free D-MEM/F-12. Each assay sample contained 300,000 CPM and 1.0 x 10^6 cells.

FIGURE 2

EFFECT OF CONCENTRATION ON THE SPECIFIC BINDING OF ^{3}H-E_2 TO MTW9/PL2 CELLS AND A SCATCHARD ANALYSIS OF THE BINDING

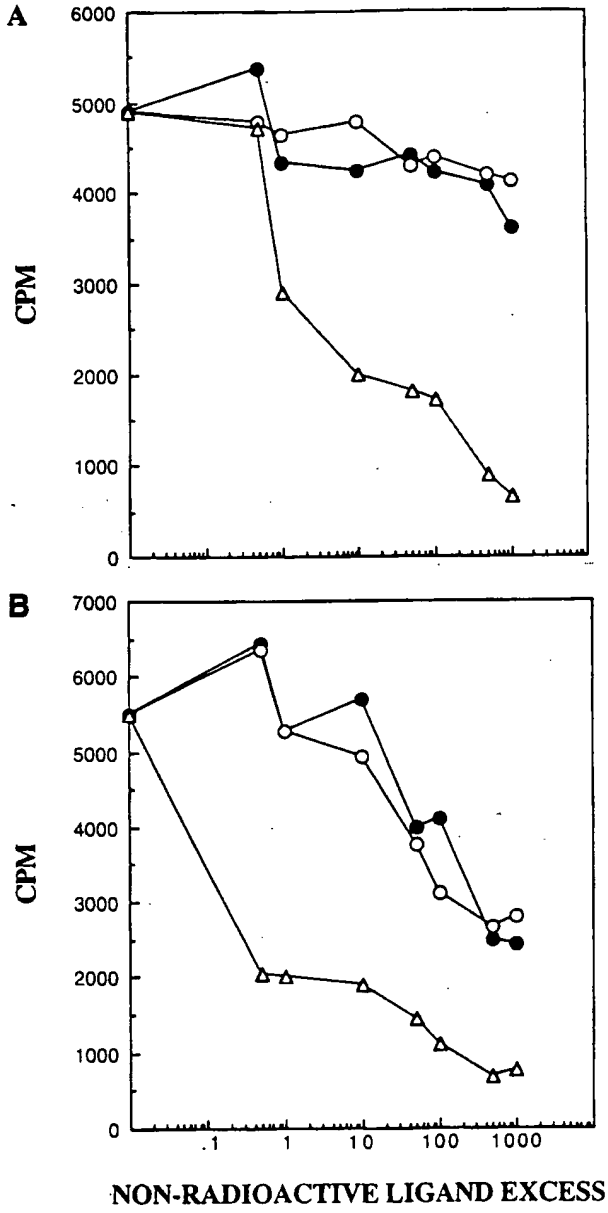


Scatchard analysis of ^{3}H-E_2 binding (closed circles) was conducted using the traditional method with labeled-unlabeled mixtures of hormone and DES (100-fold excess) over the concentration range 37 pM to 5.0 nM ^{3}H-E_2 . In both experiments, 5 nM ^{3}H-E_2 was 300,000 DPM. Each assay sample contained 1.0 x 10^6 cells.

INSERT: The insert shows a separate experiment in which the effect of ^{3}H-E_2 concentration was measured on specific binding (DPM) after 2 h at 37 ^{o}C in phenol red-free D-MEM/F-12.

FIGURE 3

EFFECT OF OTHER STEROID HORMONES ON THE BINDING OF ^3H-E_2 TO MTW9/PL2 CELLS

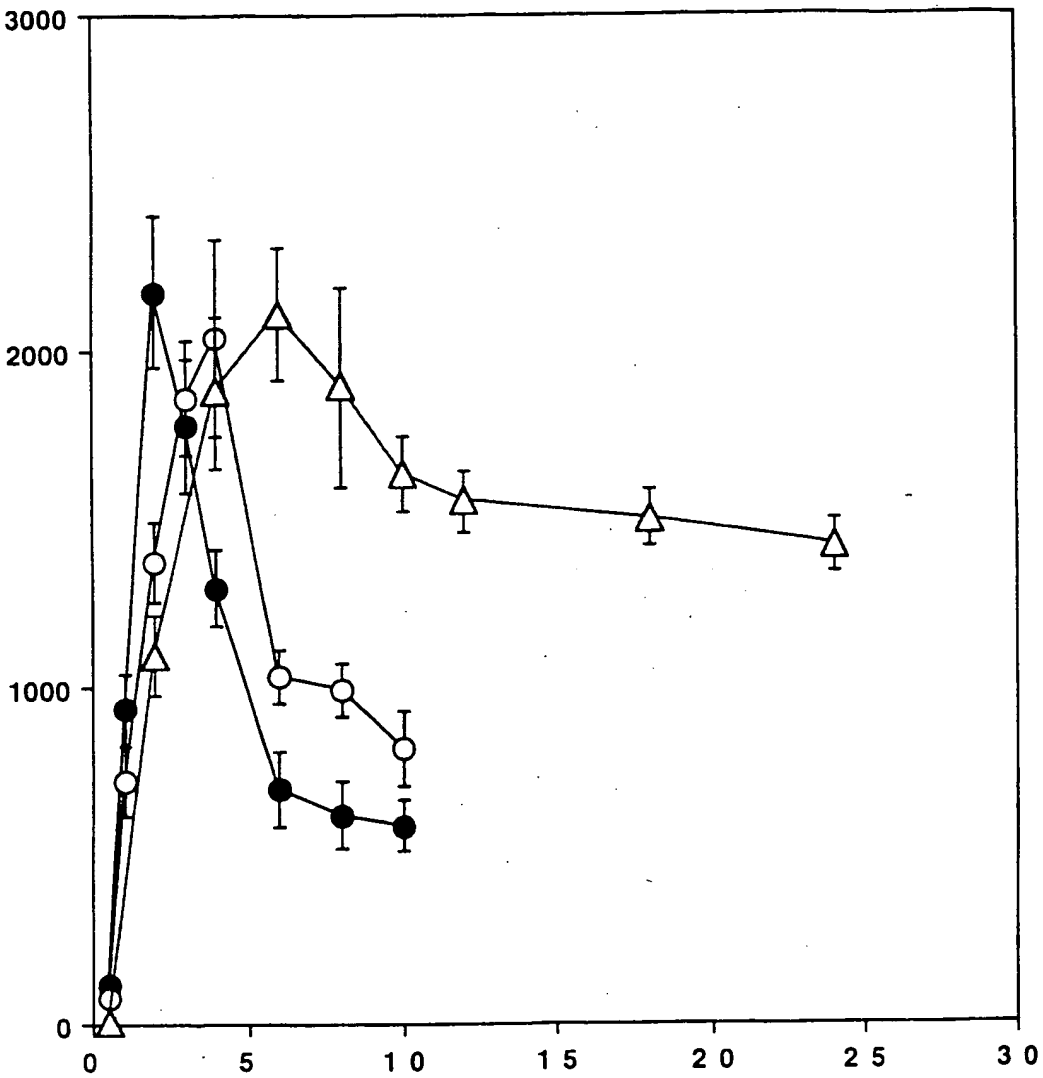


(A) shows the effects of unlabeled DES (open triangles), unlabeled DHT (open circles), and unlabeled T (closed circles).

(B) shows the effects of unlabeled DES (open triangles), unlabeled progesterone (open circles), and unlabeled cortisol (closed circles).

FIGURE 4

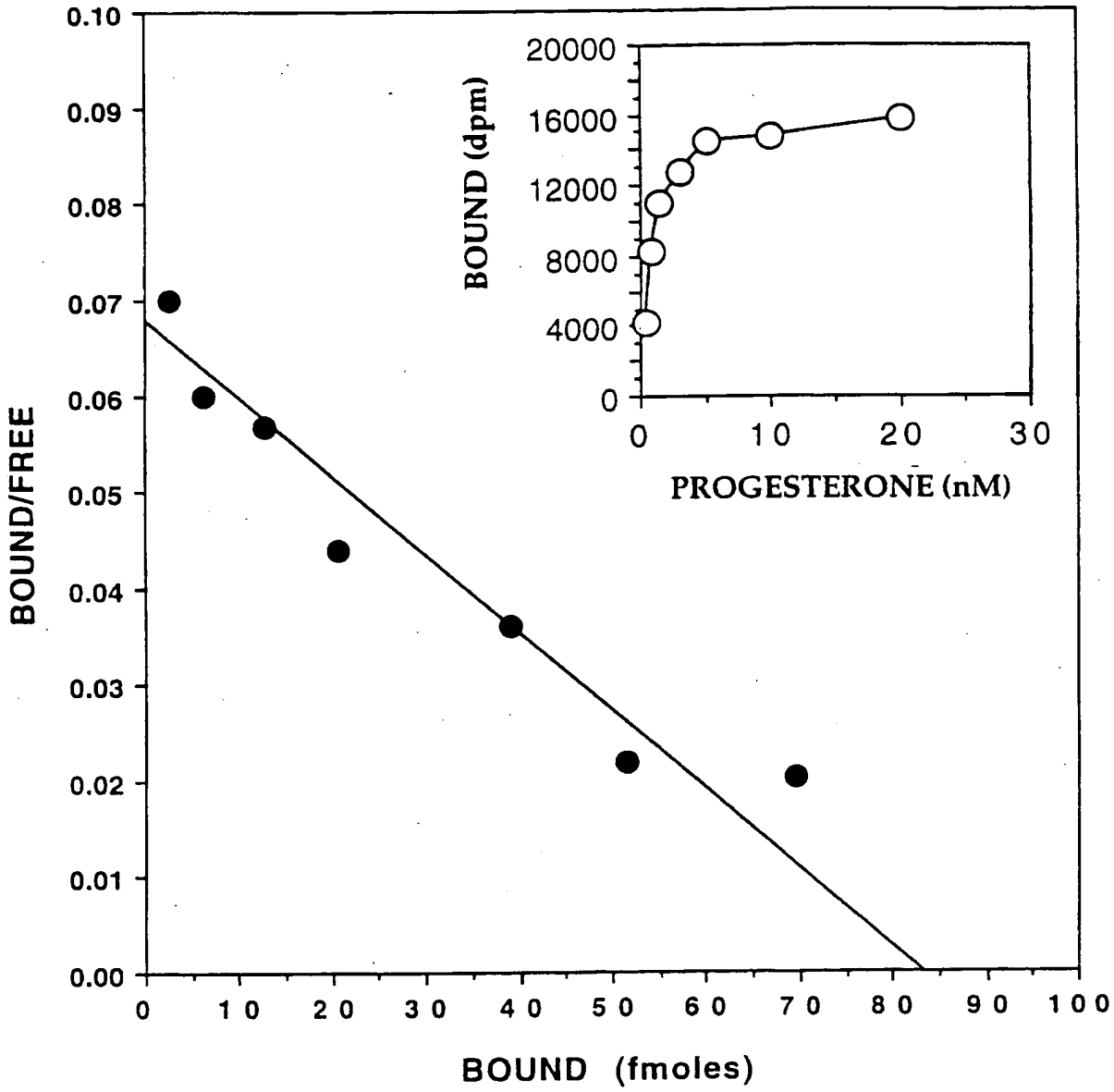
EFFECT OF TEMPERATURE ON THE SPECIFIC BINDING OF 5 nM ^{3}H-PROGESTERONE TO MTW9/PL2 CELLS



The kinetics are shown (SD of triplicates) at 37° C (closed circles), 23° C (open circles), and at 4° C (open triangles). Specific binding was determined in phenol red-free D-MEM/F-12. Each assay sample contained 287,000 CPM ^{3}H-progesterone and 1.0 x 10^{6} cells.

FIGURE 5

EFFECT OF CONCENTRATION ON THE SPECIFIC BINDING OF ^{3}H-PROGESTERONE TO MTW9/PL2 CELLS

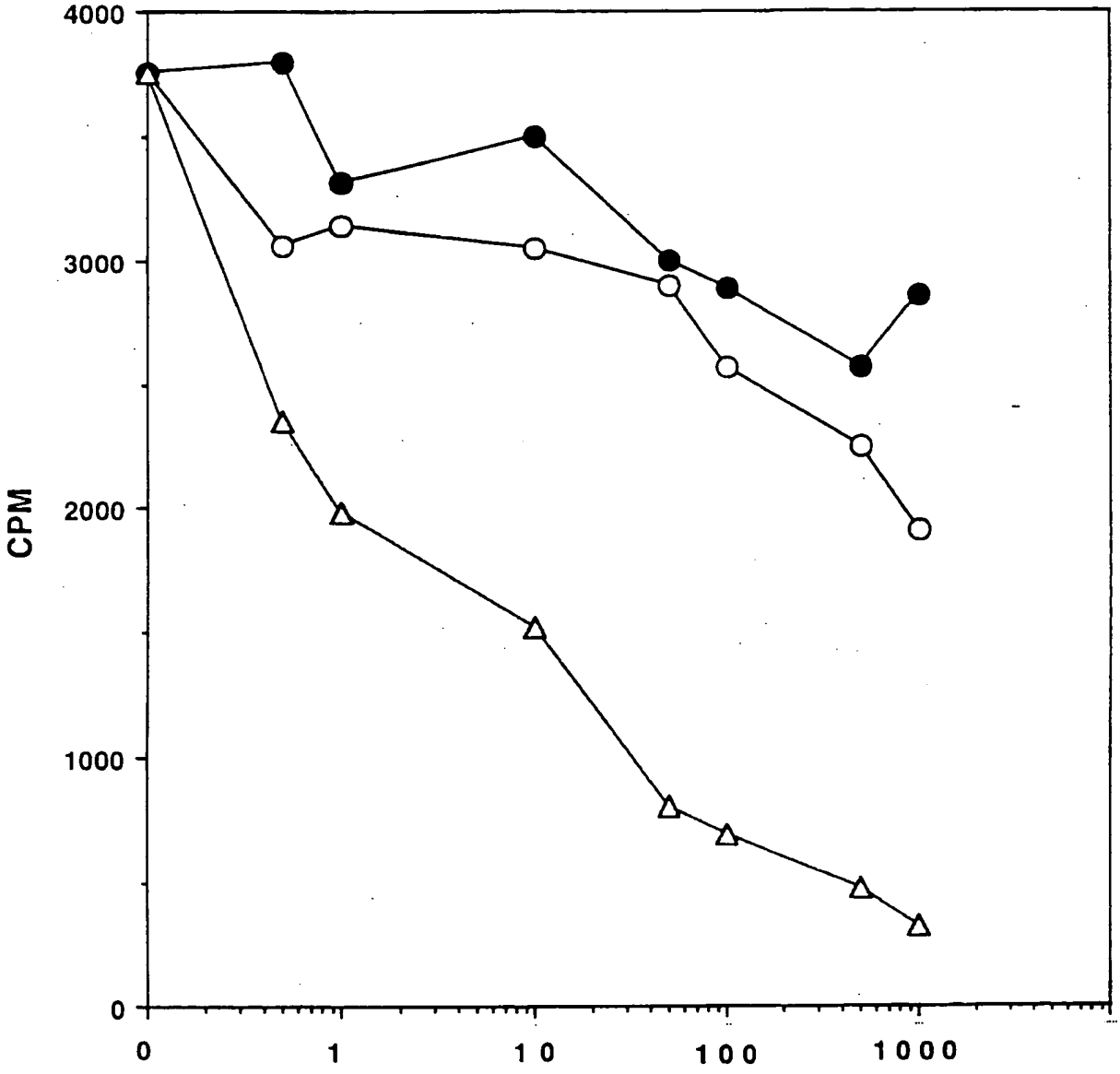


A Scatchard analysis of ^{3}H-progesterone binding (closed circles) was conducted using the traditional method with labeled-unlabeled mixtures of hormone and R5020 (100 fold excess) over the concentration range 37 pM to 5.0 nM ^{3}H-progesterone. In both experiments, 5.0 nM ^{3}H-progesterone was 287,000 CPM. Each assay sample contained 1.0 x 10^6 cells.

INSERT: The insert shows a separate experiment in which the effect of ^{3}H-progesterone concentration was measured on specific binding (bound dpm) after 2 h at 37° C in phenol red-free D-MEM/F-12.

FIGURE 6

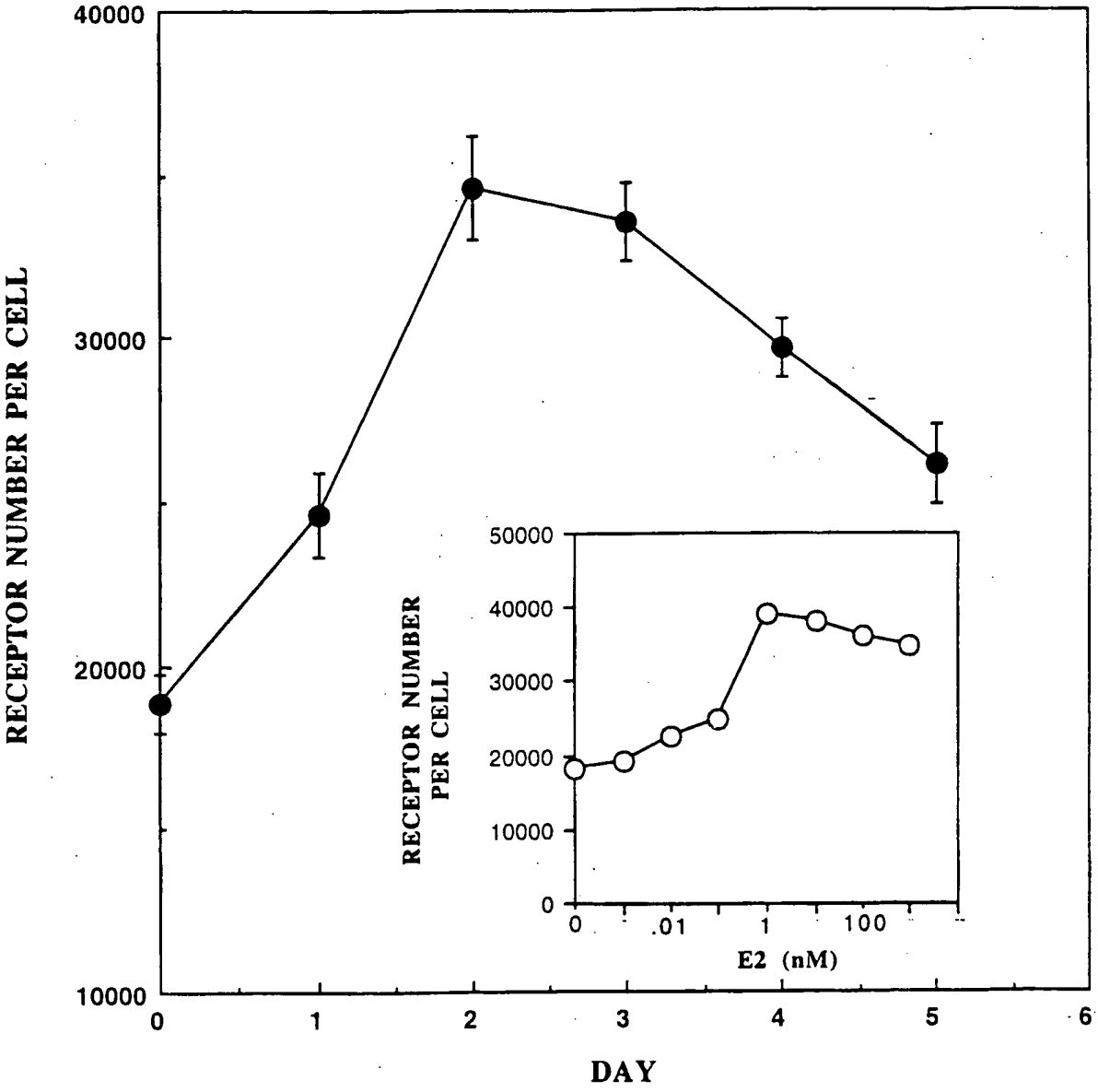
EFFECT OF STEROID HORMONES ON THE BINDING OF ^{3}H-PROGESTERONE TO MTW9/PL2 CELLS



The cells were incubated at 37 ^{o} C for 2 h in the presence of 5 nM ^{3}H-progesterone (287,000 CPM) alone or in the presence of the labeled hormone plus the designated fold excess (M) of unlabeled R5020 (open triangles), unlabeled DHT (open circles), or unlabeled T (closed circles). Each assay sample contained 1.0 x 10 ^{6} cells.

FIGURE 7

EFFECT OF E_2 ON THE PROGESTERONE RECEPTOR CONTENT OF MTW9/PL2 CELLS



Each specific binding presented is the average of triplicate incubations ± SD (closed circles).

INSERT: The insert shows the effect of E_2 concentration in the culture medium for 2 d prior to the assay of progesterone receptors (open circles).

FIGURE 8

WESTERN IMMUNOBLOTTING OF STEROID HORMONE RECEPTORS

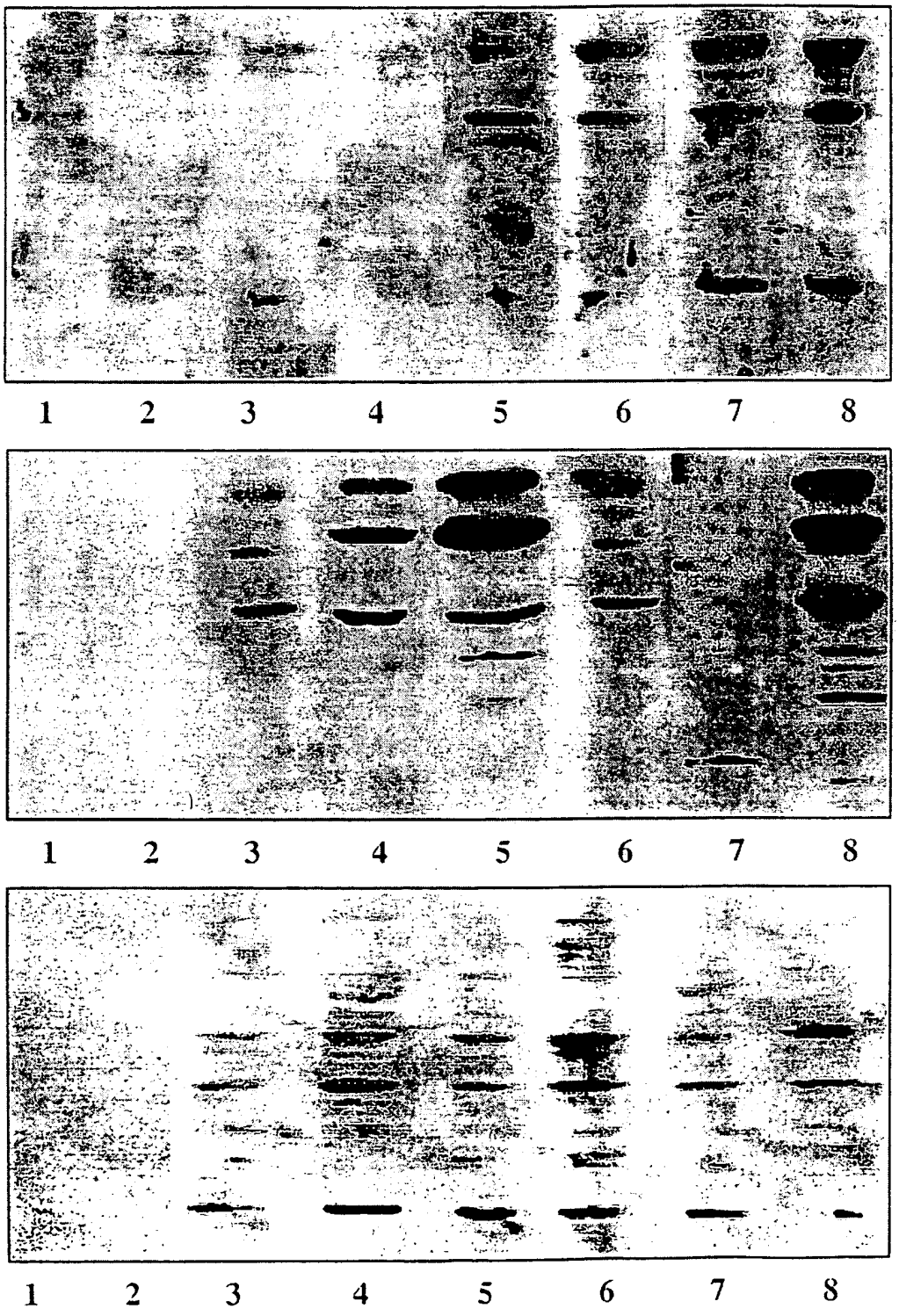
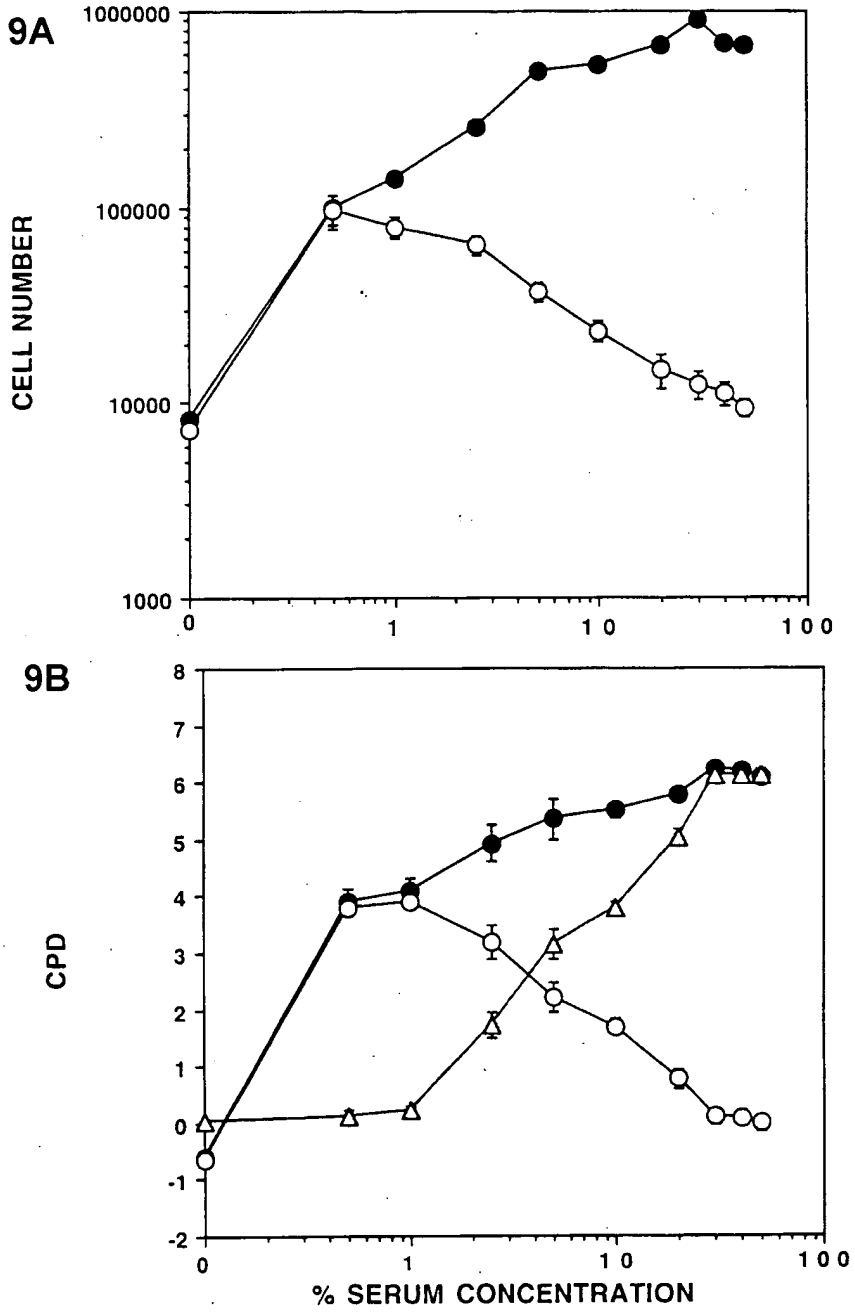


FIGURE 9

MTW9/PL2 CELL GROWTH IN 50% CDE - HORSE SERUM

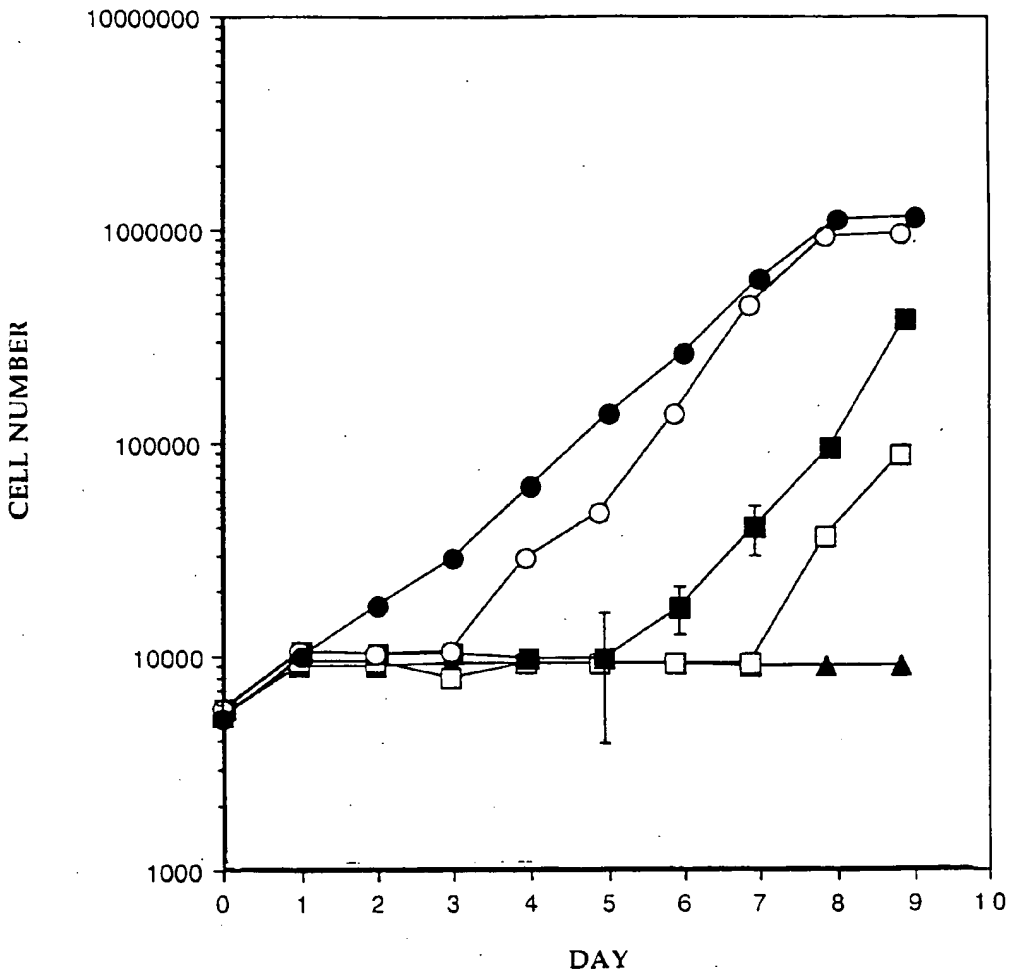


A: DATA EXPRESSED AS CELL NUMBER AFTER 7 DAYS
Growth with 1.0 x 10 M E (closed circles) and without hormone (open circles) in medium containing the designated concentrations of serum.

B. DATA IN (A) EXPRESSED AS CPD
The symbols indicate the same conditions as (A) except the open triangles show CPD differences between growth in dishes with and without the hormone (Difference = estrogenic effect on growth).

FIGURE 10

MTW9/PL2 CELL GROWTH IN 50% CDE - HORSE SERUM WITH ESTROGENS ADDED AT VARIOUS TIMES AFTER SEEDING

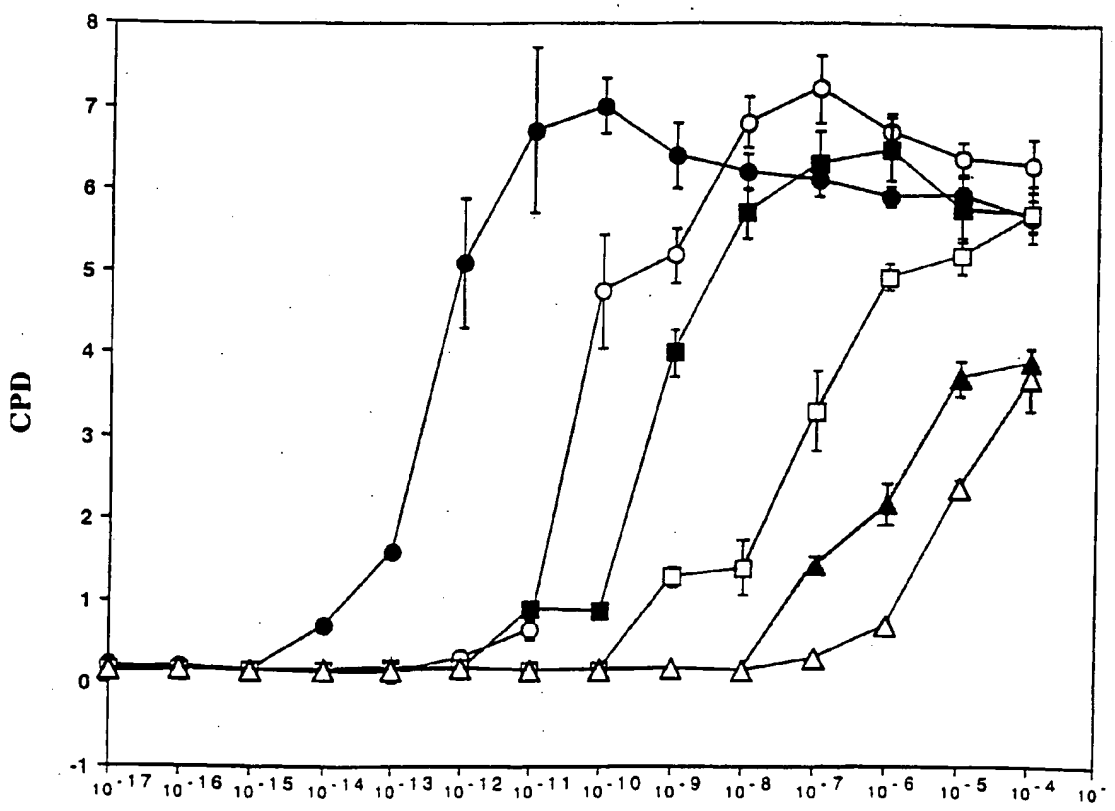


LEGEND:

Control growth in the absence of exogenous estrogen is shown by (triangles). In other dishes, 1.0 x 10^{-8} M E_2 was added at the beginning of the experiment (closed circles), after 48 h (open circles), after 96 h (closed squares), or after 144 h (open squares).

FIGURE 11

STEROID HORMONE DOSE RESPONSE EFFECTS WITH MTW9/PL2 CELLS IN 50% CDE - HORSE SERUM

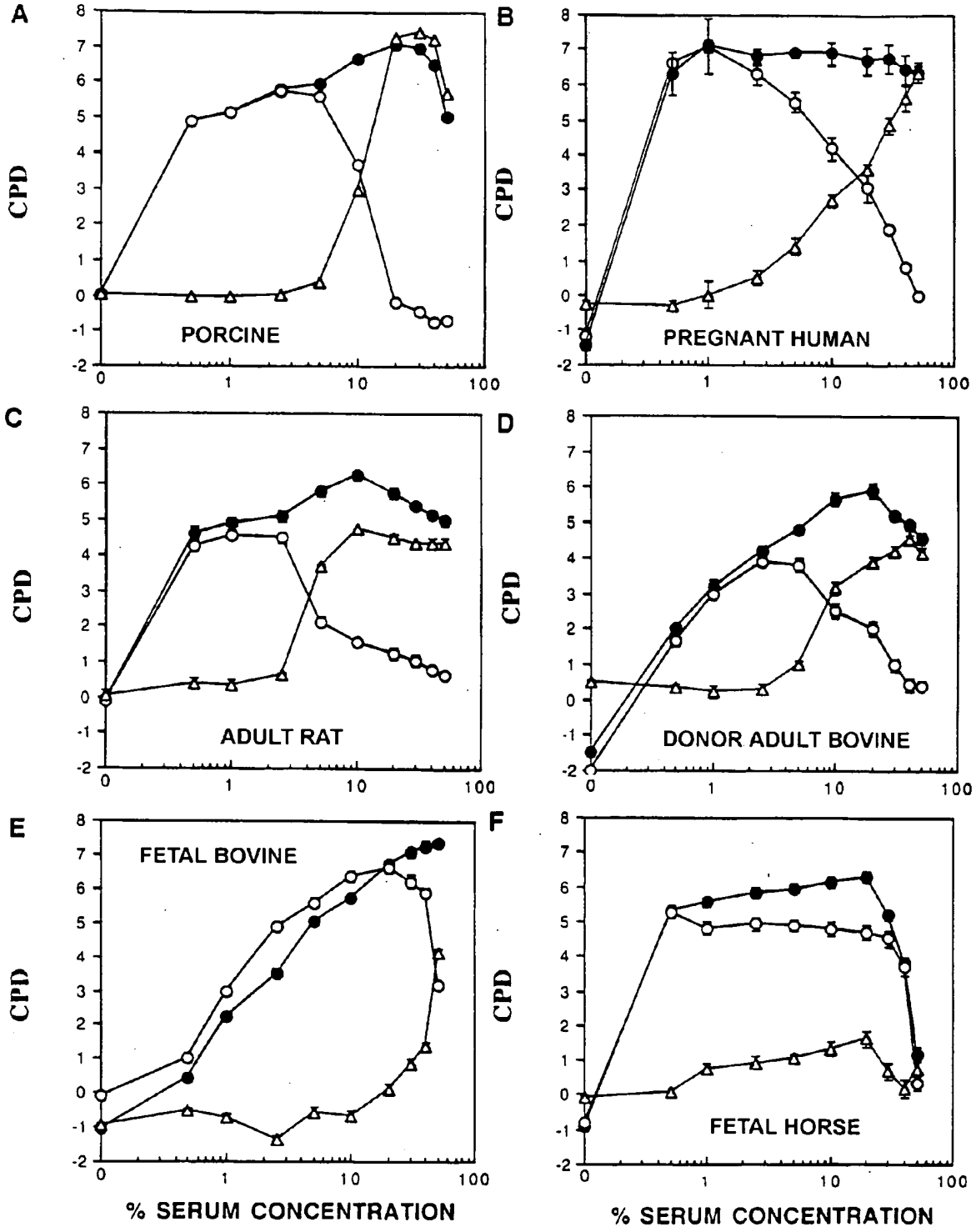


LEGEND:

Closed circles = E_2
Open circles = E_1
Closed squares = E_3
Open squares = Progesterone
Closed triangles = DHT
Open triangles = T

FIGURE 12

MTW9PL2 CELL GROWTH IN CDE SERUM FROM DIFFERENT SPECIES



LEGEND: Open circles = $-E_2$
Closed circles = $+E_2$
Open triangles = Estrogenic effect

FIGURE 13

CDE HORSE SERUM TITRATION
GH4C1 CELLS

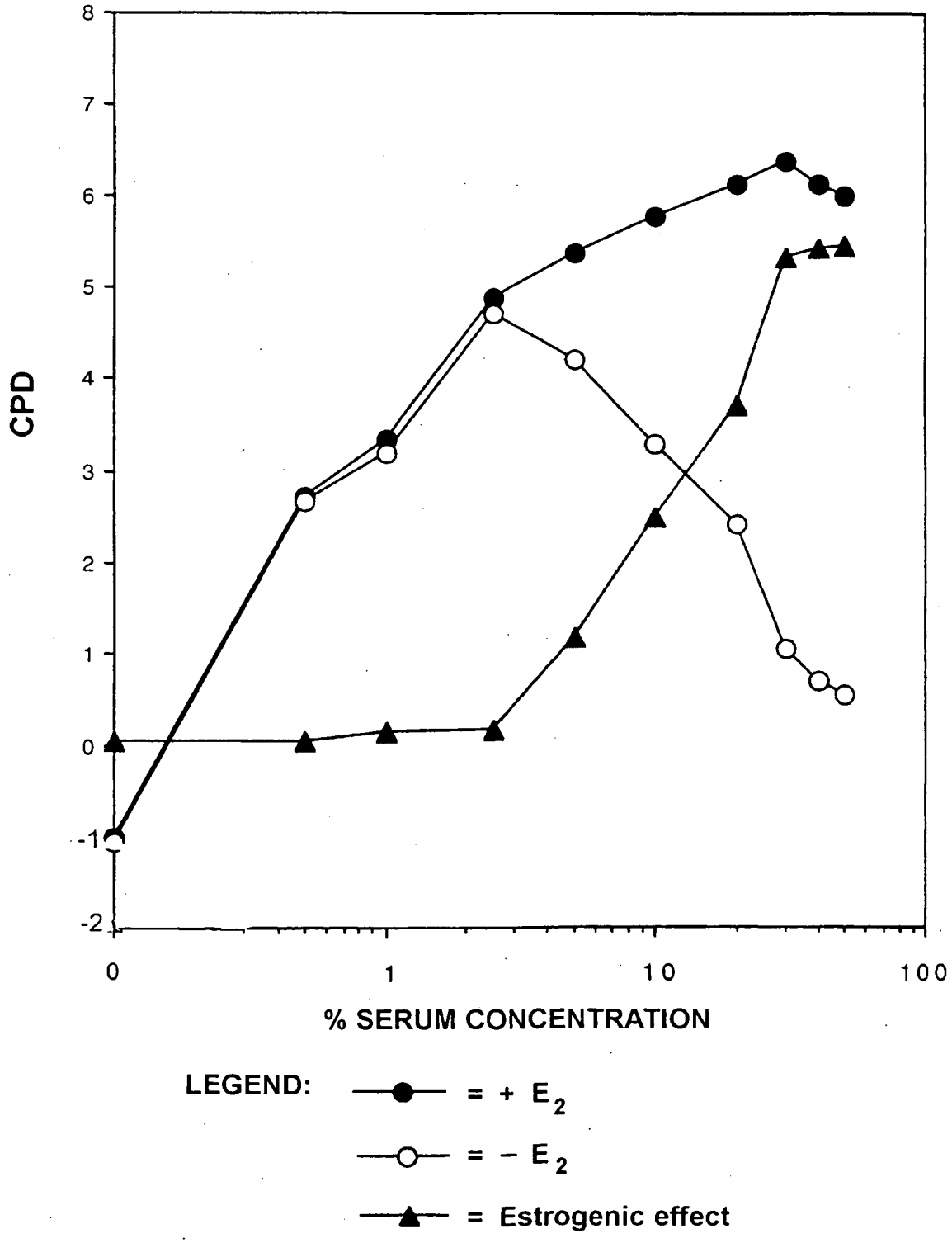
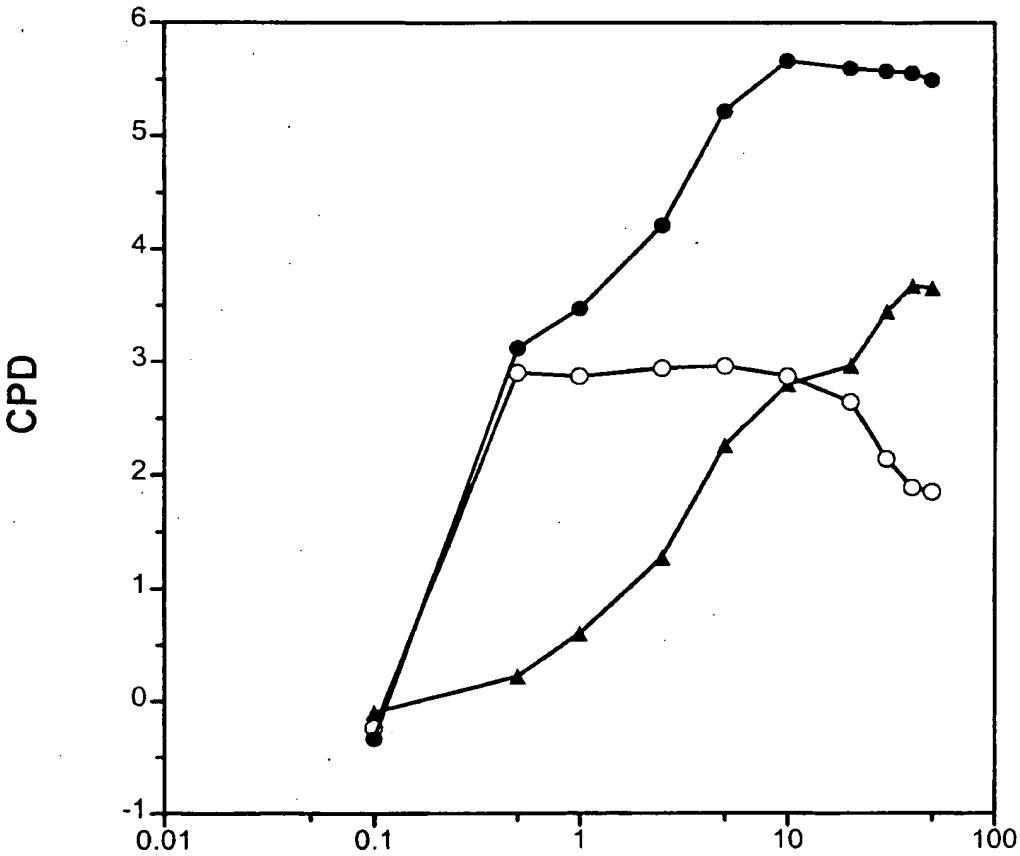


FIGURE 14

ZR-75-1 CELLS IN CDE - HORSE SERUM ± 10 nM E_2

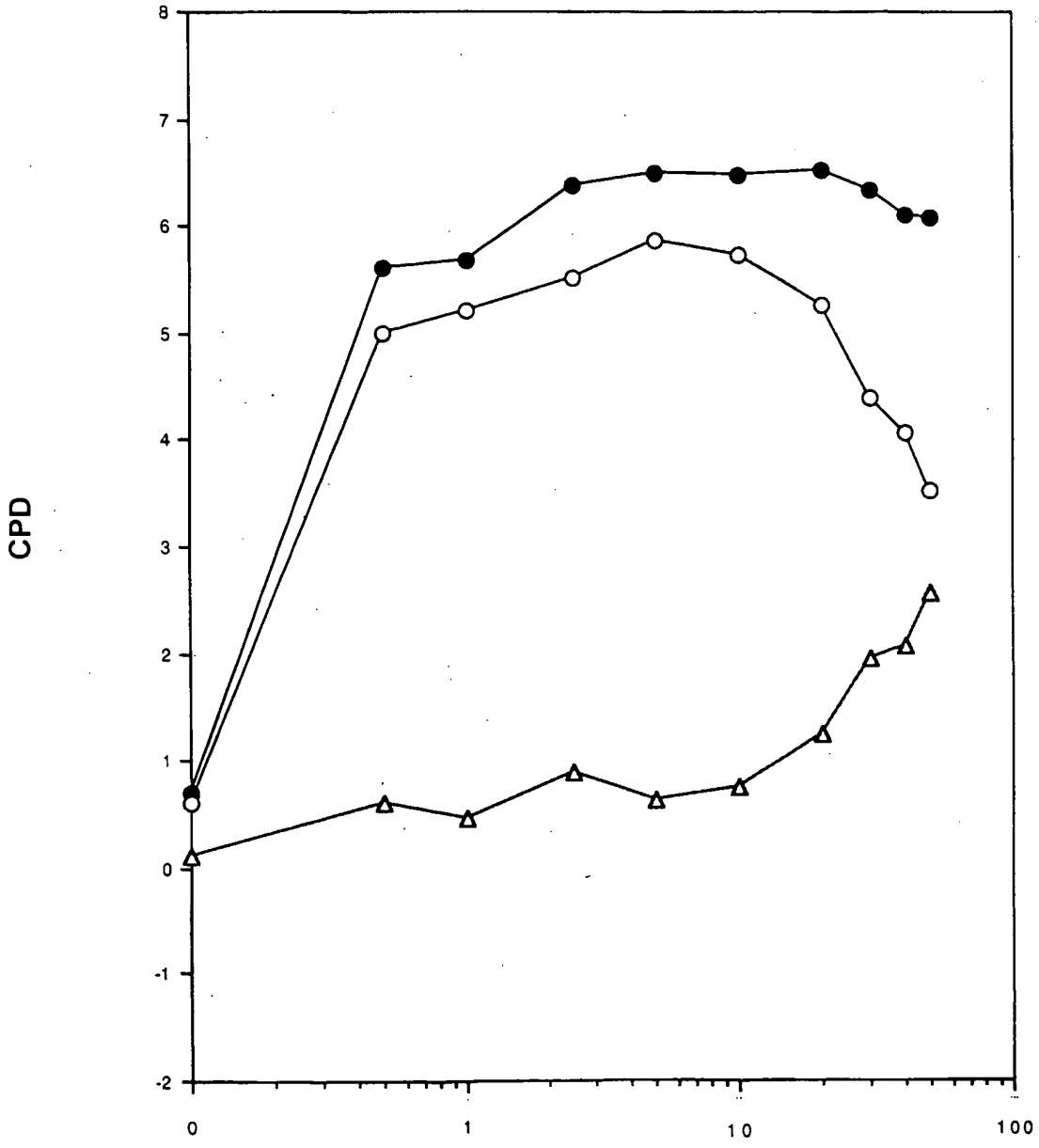


LEGEND:

Closed circles = $+E_2$
Open circles = $-E_2$
Closed triangles = Estrogenic effect

FIGURE 15

MCF7A CELL GROWTH IN CDE - HORSE SERUM $\pm E_2$

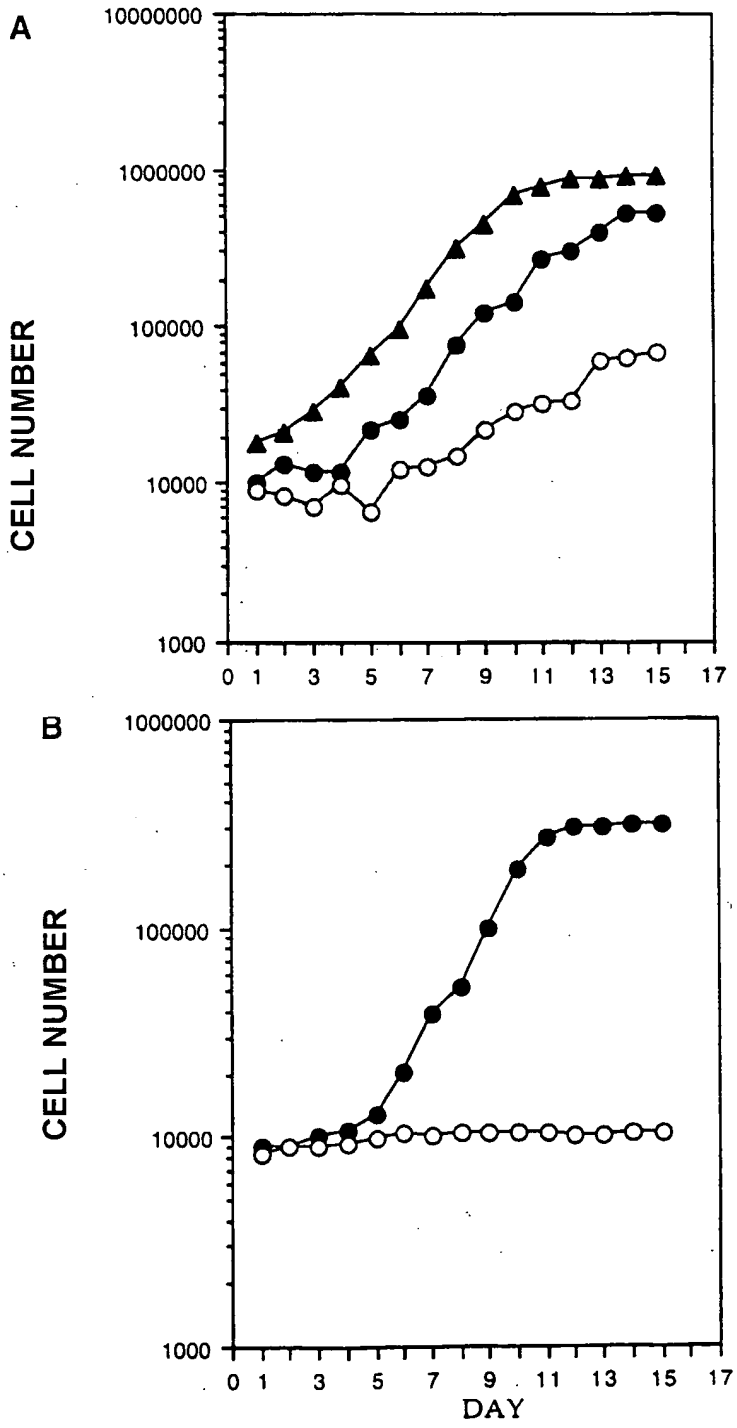


LEGEND:

Closed circles = $+E_2$
Open circles = $-E_2$
Closed triangles = Estrogenic effect

FIGURE 16

GROWTH KINETICS OF T47D HUMAN BREAST CANCER CELLS IN CDE - HORSE SERUM ±10 nM E_2

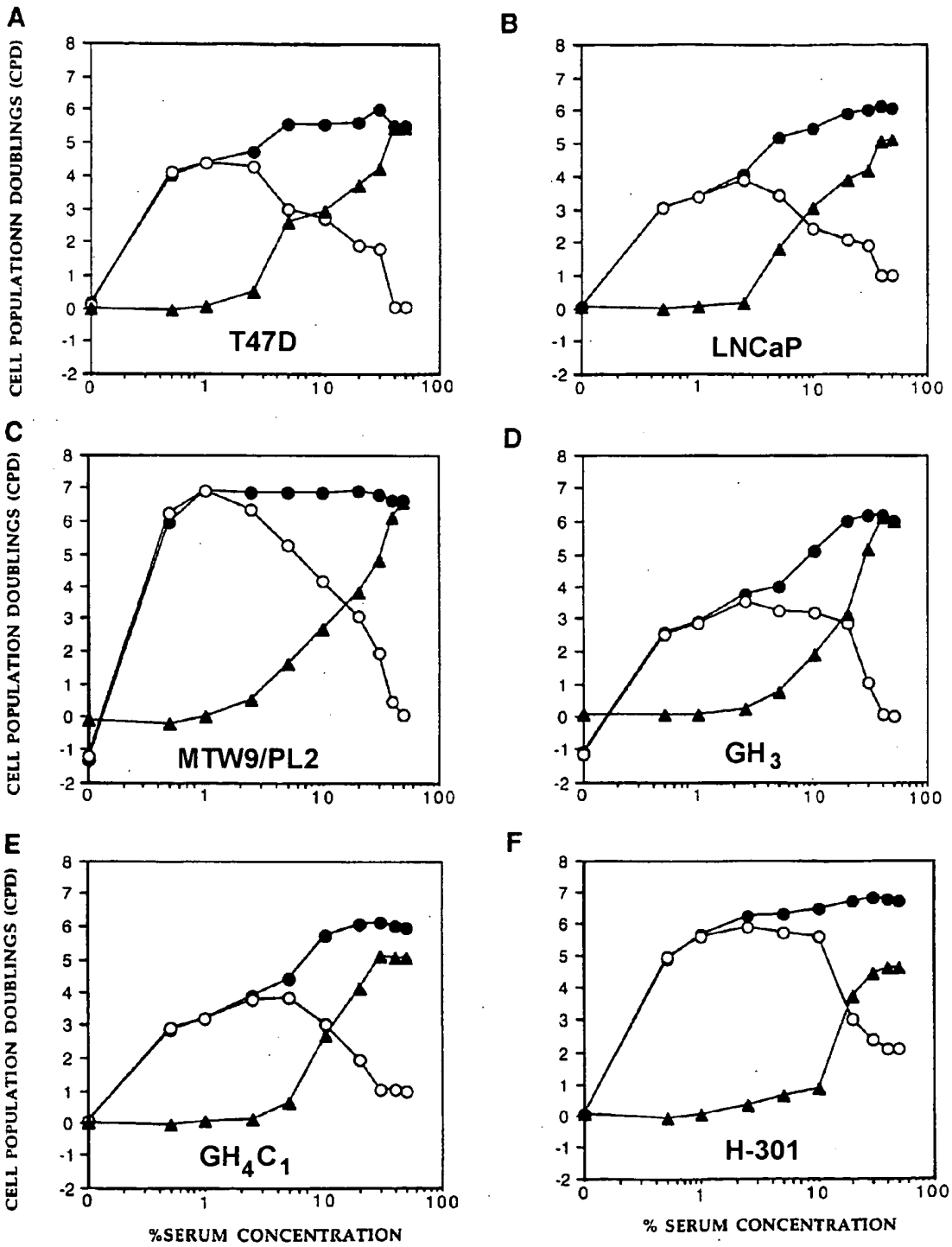


(A) The growth of the cells in medium with 20% (v/v) serum with 10 nM E_2 (closed circles) and without the steroid (open circles). As comparison, growth is shown in medium containing 10% (v/v) FBS (triangles).

(B) T47D cell growth kinetics in medium with 50% (v/v) serum with E_2 (closed circles) and without the steroid (open circles).

FIGURE 17

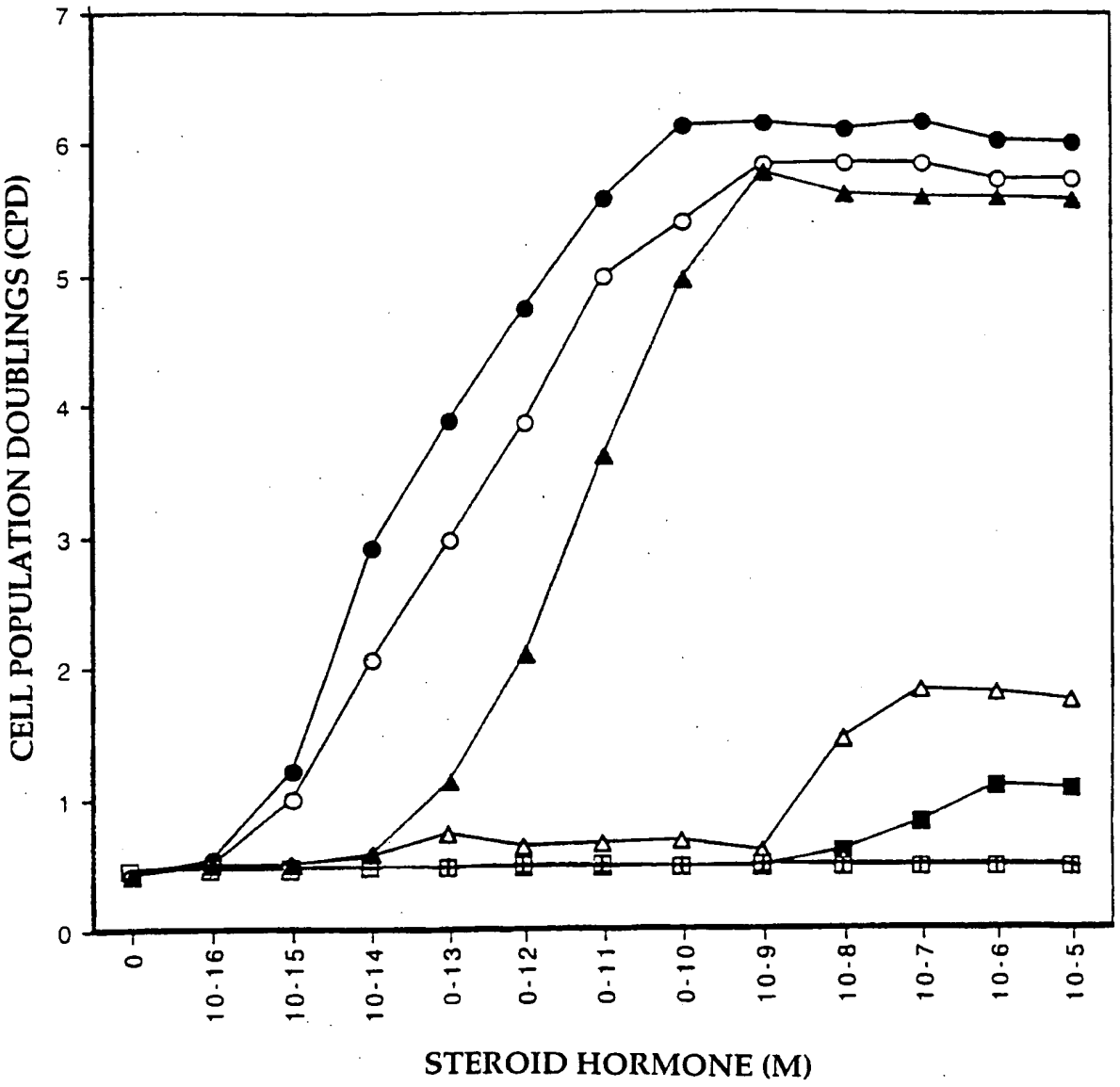
GROWTH OF HUMAN & RODENT CELL LINES IN 50% CDE - HORSE SERUM $\pm E_2$ (10 nM)



LEGEND: Closed circles = Medium with 10 nM E_2
Open circles = Medium without E_2
Triangles = Estrogenic effect

FIGURE 18

DOSE RESPONSE OF STEROID HORMONES WITH T47D CELLS IN 50% CDE - HORSE SERUM

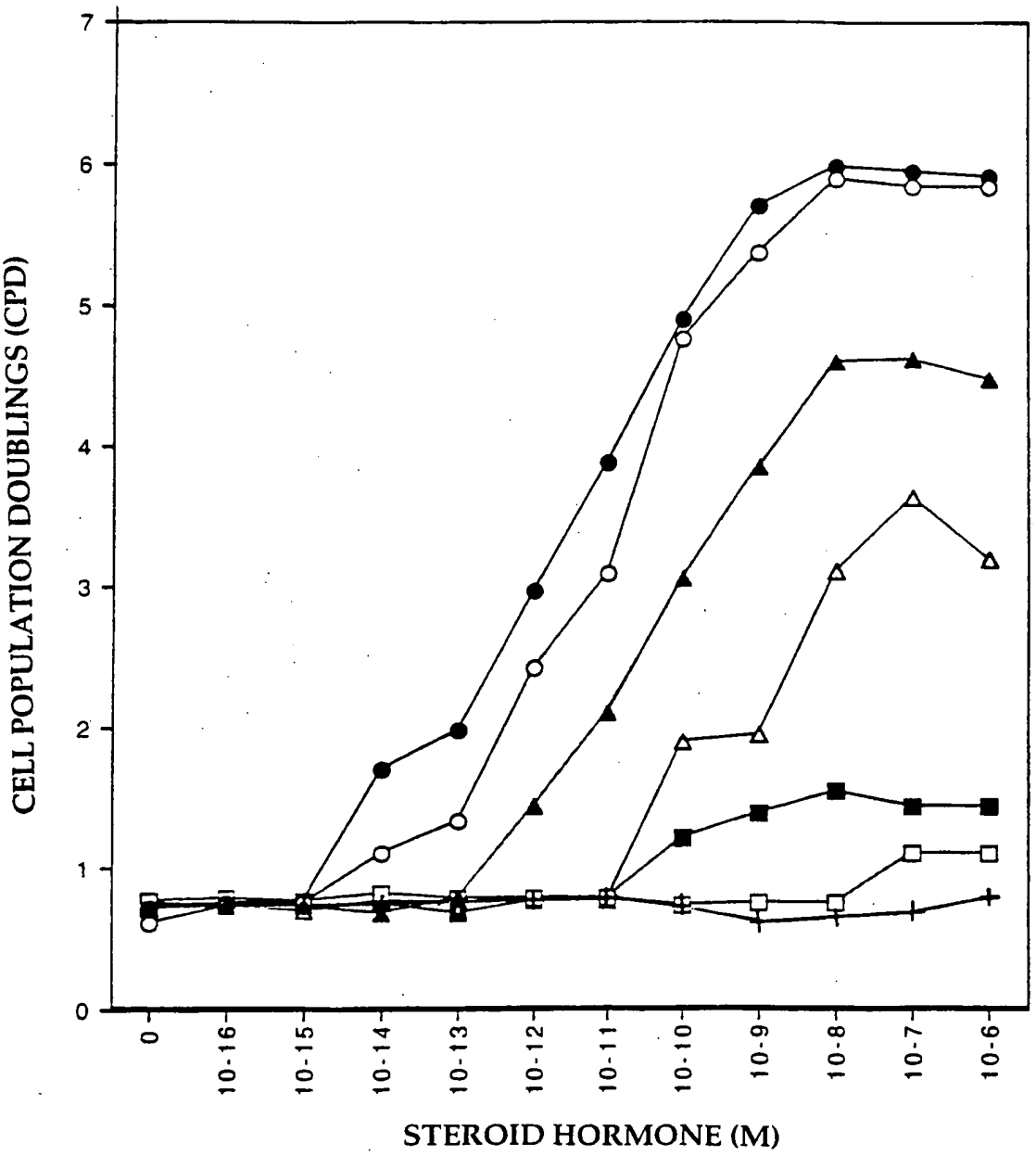


LEGEND:

Growth after 14 days is shown in response to:
- **Closed circles = E_2**
- **Open circles = E_1**
- **Closed triangles = E_3**
- **Open triangles = DHT**
- **Closed squares = Testosterone**
- **Open squares = Progesterone**
- **Crosses = Cortisol**

FIGURE 19

DOSE RESPONSE OF STEROID HORMONES WITH GH_4C_1 CELLS IN 50% CDE - HORSE SERUM



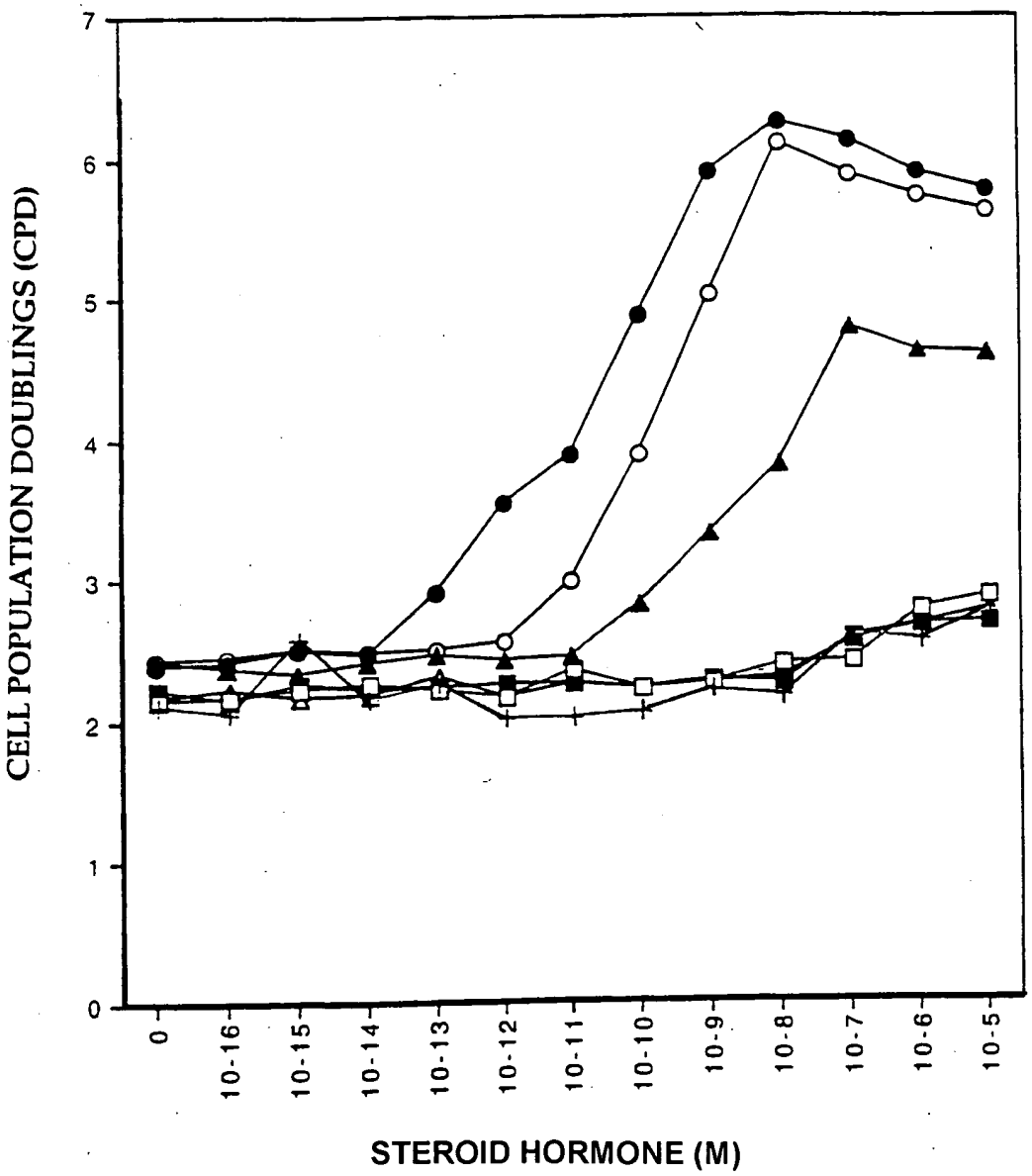
LEGEND:

Growth after 11 days is shown in response to:

Closed circles = E_2
Open circles = E_1
Closed triangles = E_3
Open triangles = DHT
Closed squares = Testosterone
Open squares = Progesterone
Crosses = Cortisol

FIGURE 20

DOSE RESPONSE OF STEROID HORMONES
WITH H-301 CELLS IN 50% CDE - HORSE SERUM

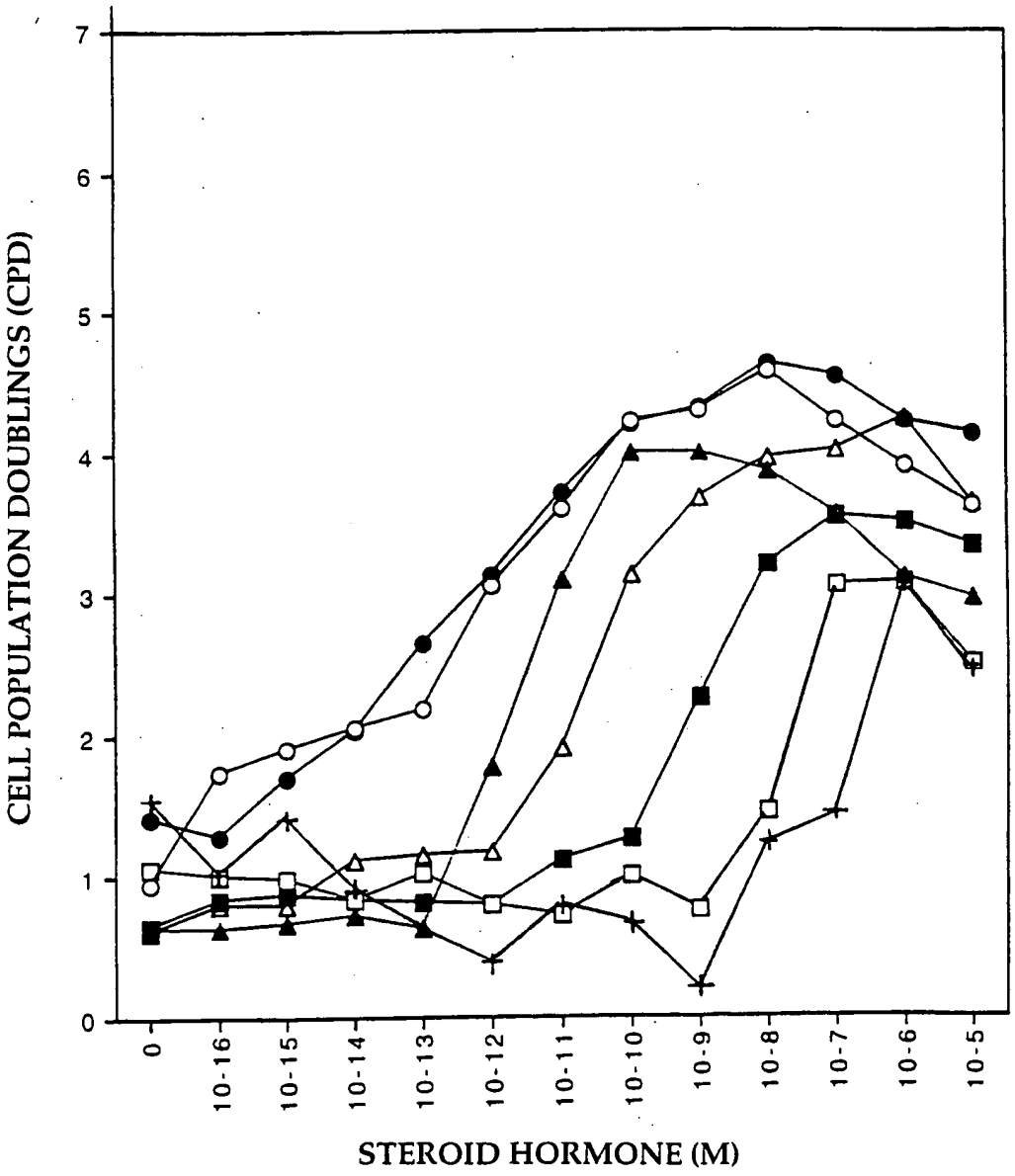


LEGEND:

Growth after 9 days is shown in response to:
Closed circles = E_2
Open circles = E_1
Closed triangles = E_3
Open triangles = DHT
Closed squares = Testosterone
Open squares = Progesterone
Crosses = Cortisol

FIGURE 21

DOSE RESPONSE OF STEROID HORMONES WITH LNCaP CELLS IN 50% CDE - HORSE SERUM



LEGEND:

Growth after 14 days is shown in response to:

- **Closed circles = E_2**
- **Open triangles = E_1**
- **Open squares = E_3**
- **Open circles = DHT**
- **Closed triangles = Testosterone**
- **Closed squares = Progesterone**
- **Crosses = Cortisol**

FIGURE 22

T_3 TITRATION OF GH_3 CELLS GROWN IN SERUM - FREE MEDIUM (PCM)

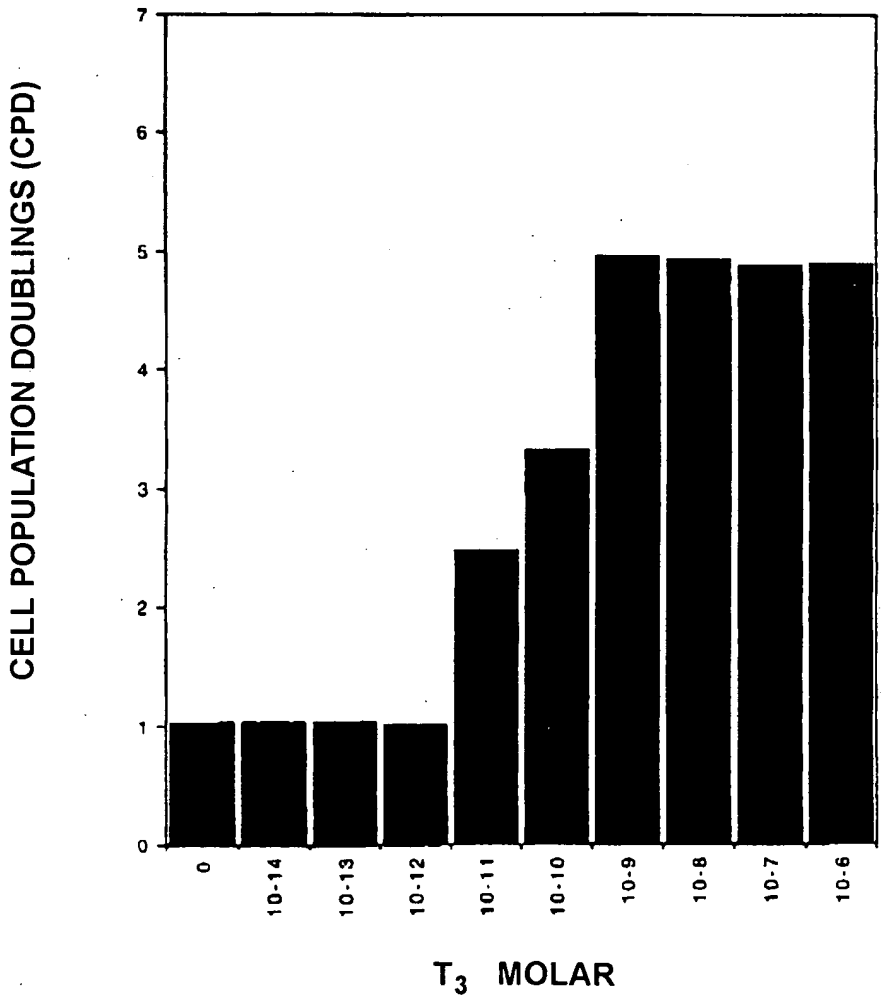


FIGURE 23

E_2 TITRATION OF GH_3 CELLS GROWN IN SERUM-FREE MEDIUM MINUS T_3

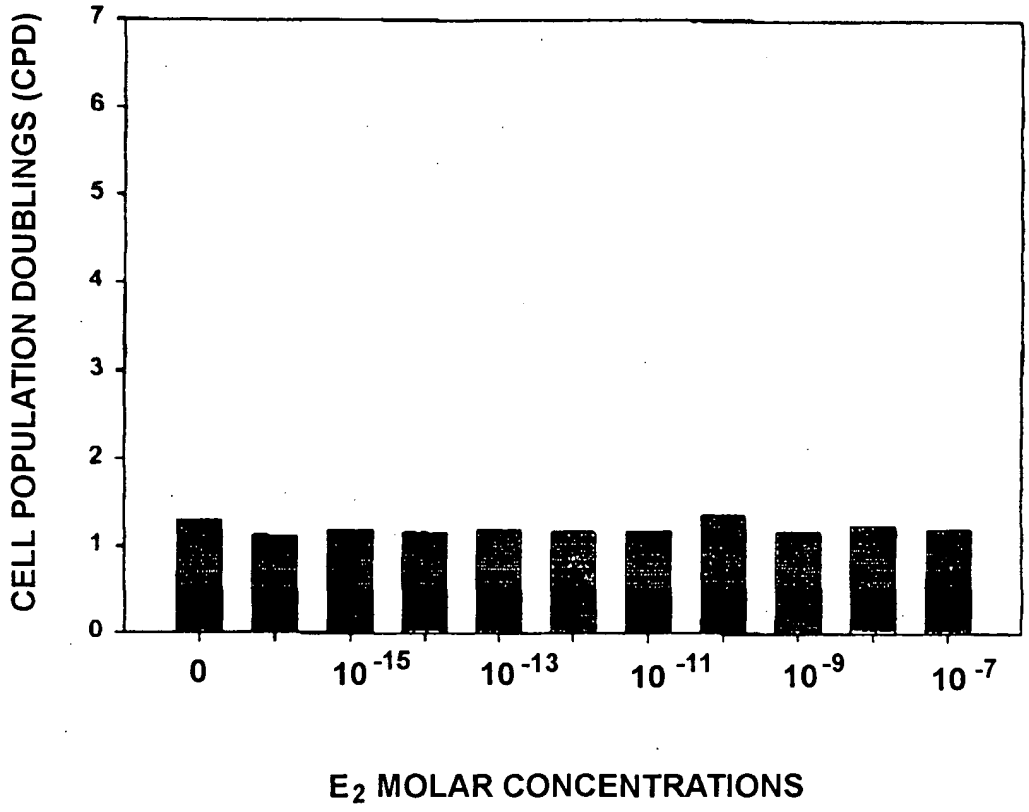


FIGURE 24

EFFECT OF T_3 ON GH CELL LINES: GROWTH IN 2.5% CDE - HORSE SERUM WITH NO E_2

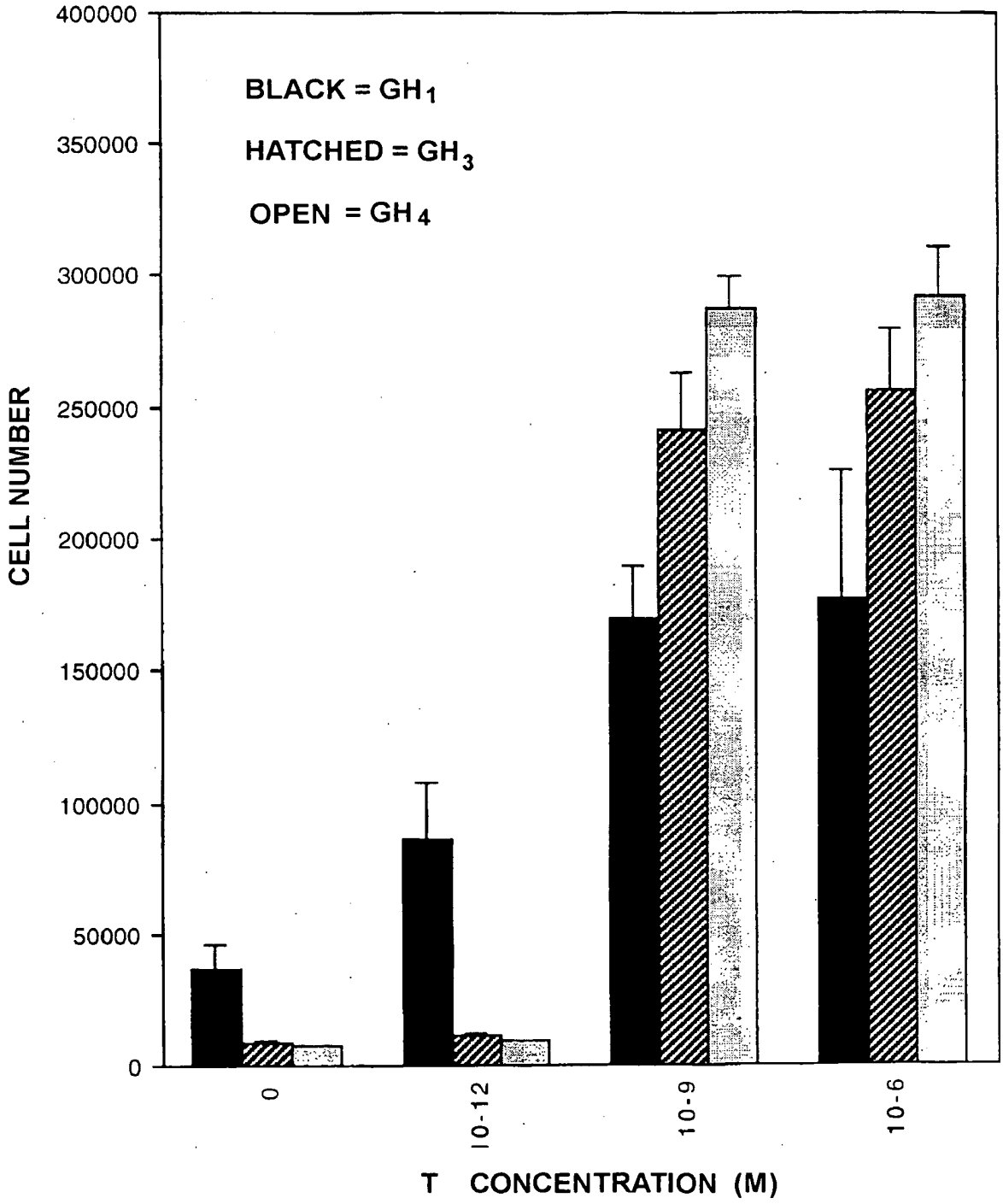


FIGURE 25

EFFECT OF T_3 ON PITUITARY CELL LINES INCUBATED IN 50% CDE - HORSE SERUM

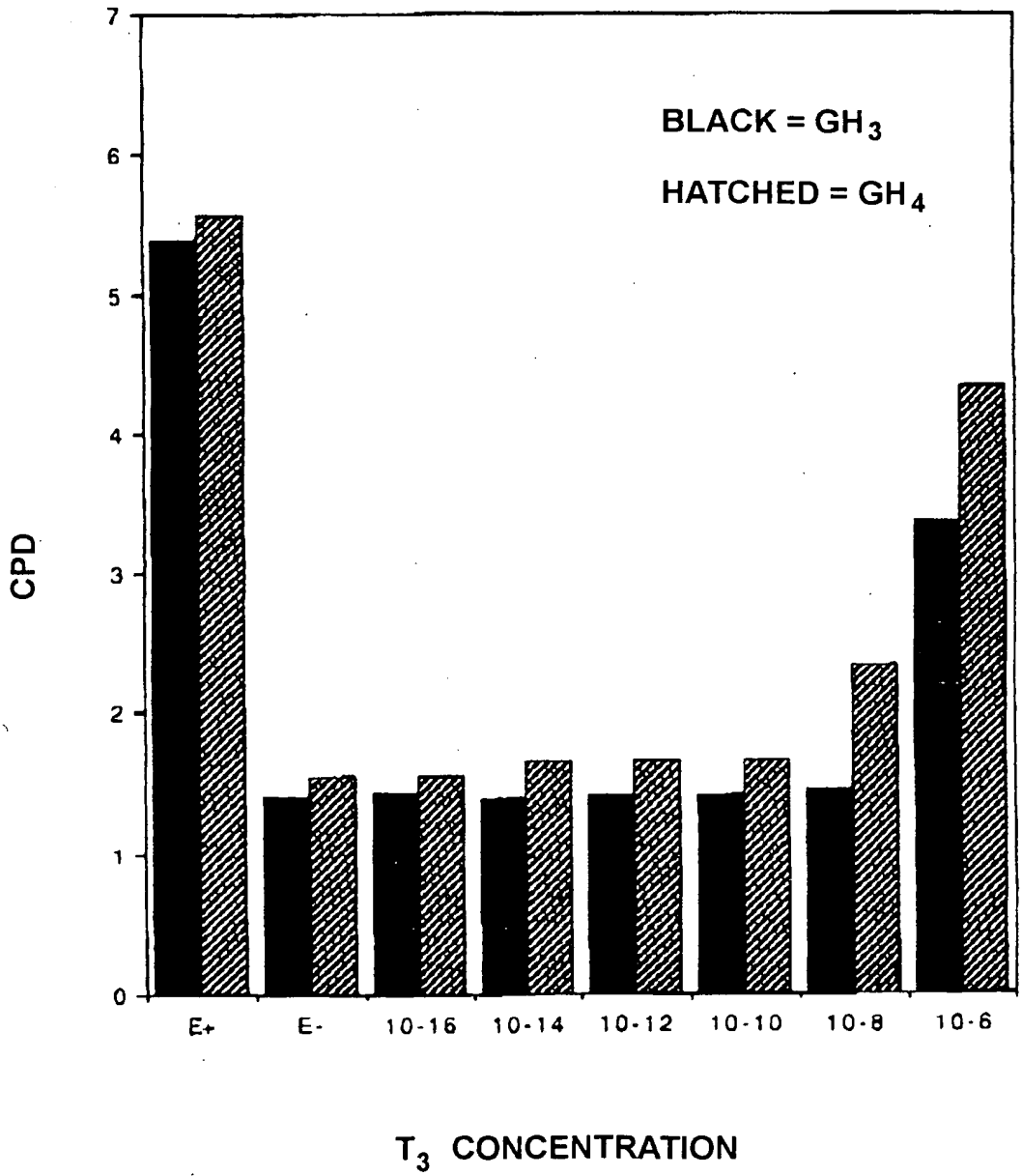
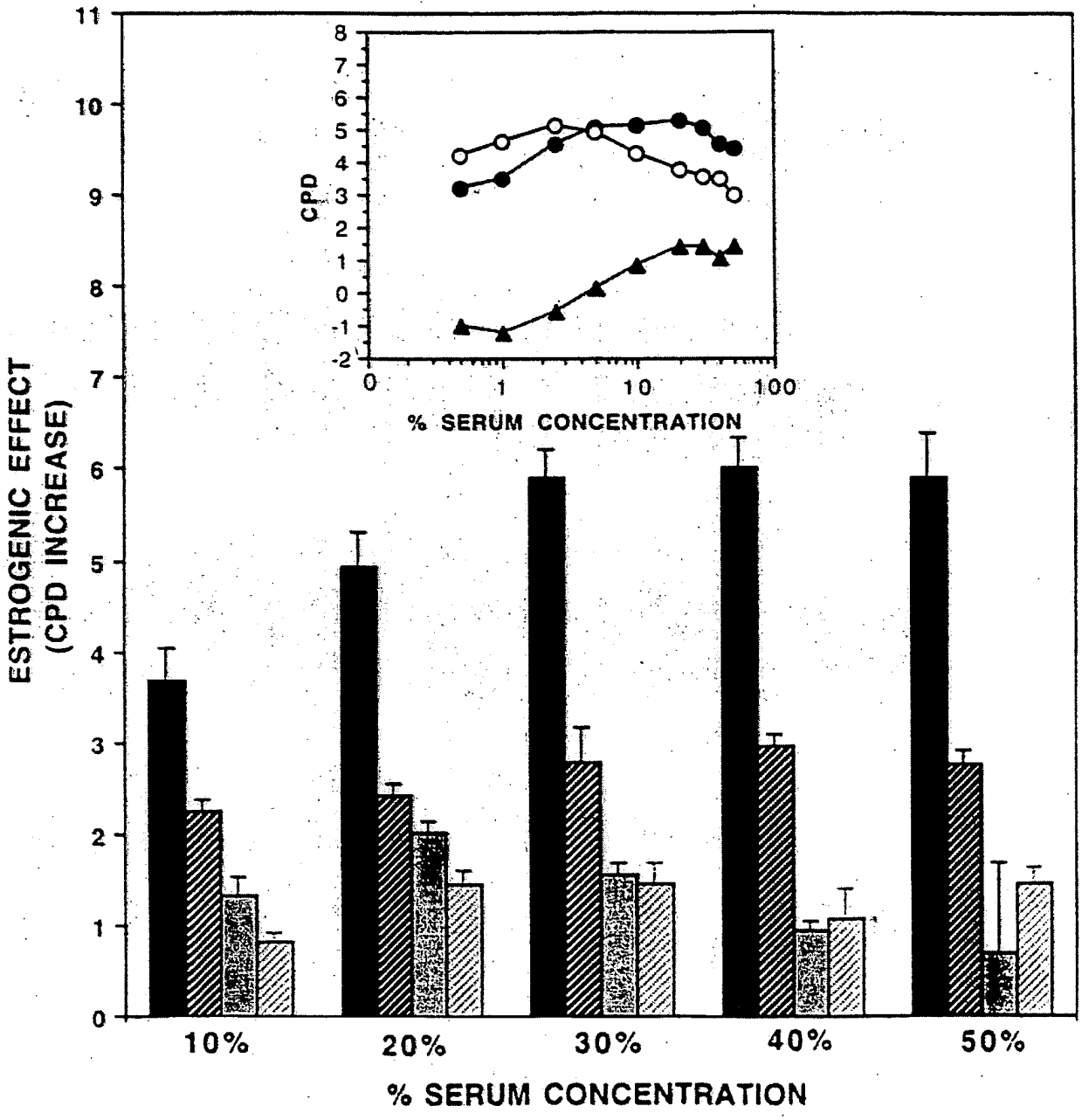


FIGURE 26

COMPARISON OF 56°C AND 34°C CHARCOAL EXTRACTED SERUM



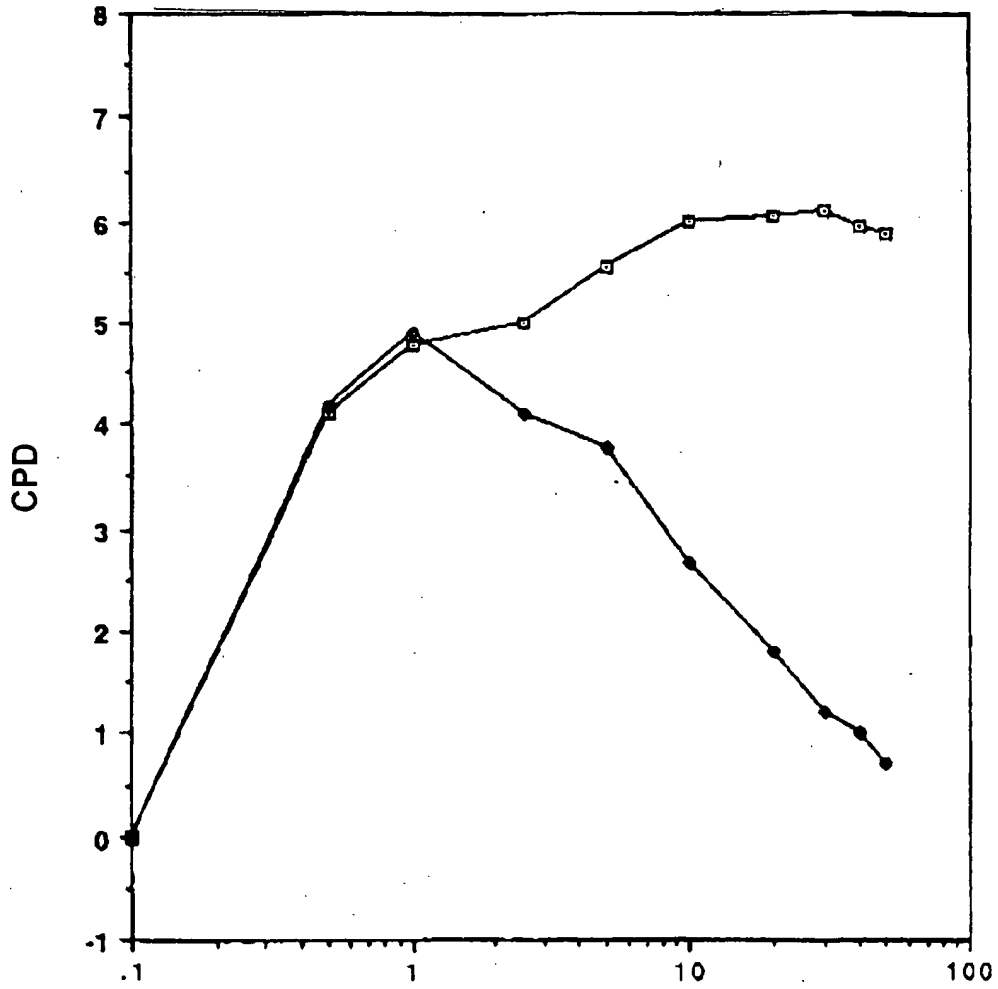
FILLED BARS: Estrogenic effect in 34°C prepared CDE-serum
DARK HATCHED BARS: 56°C prepared CDE-serum
LIGHT SHADED BARS: Charcoal extracted at 34°C then charcoal extraction at 56°C
LIGHT HATCHED BARS: Charcoal extracted at 34°C then incubation for 20 min at 56°C

INSERT: Dose-response growth effects of horse serum extracted at 34°C followed by incubation for 20 min at 56°C

Open circles - Growth without E_2
Closed circles - Growth with 1.0 x 10^{-8} M E_2
Triangles - Estrogenic effect

FIGURE 27

HORSE SERUM TITRATION WITH MTW9/PL2 CELLS EXTRACTION BY XAD-4 RESIN



LEGEND:

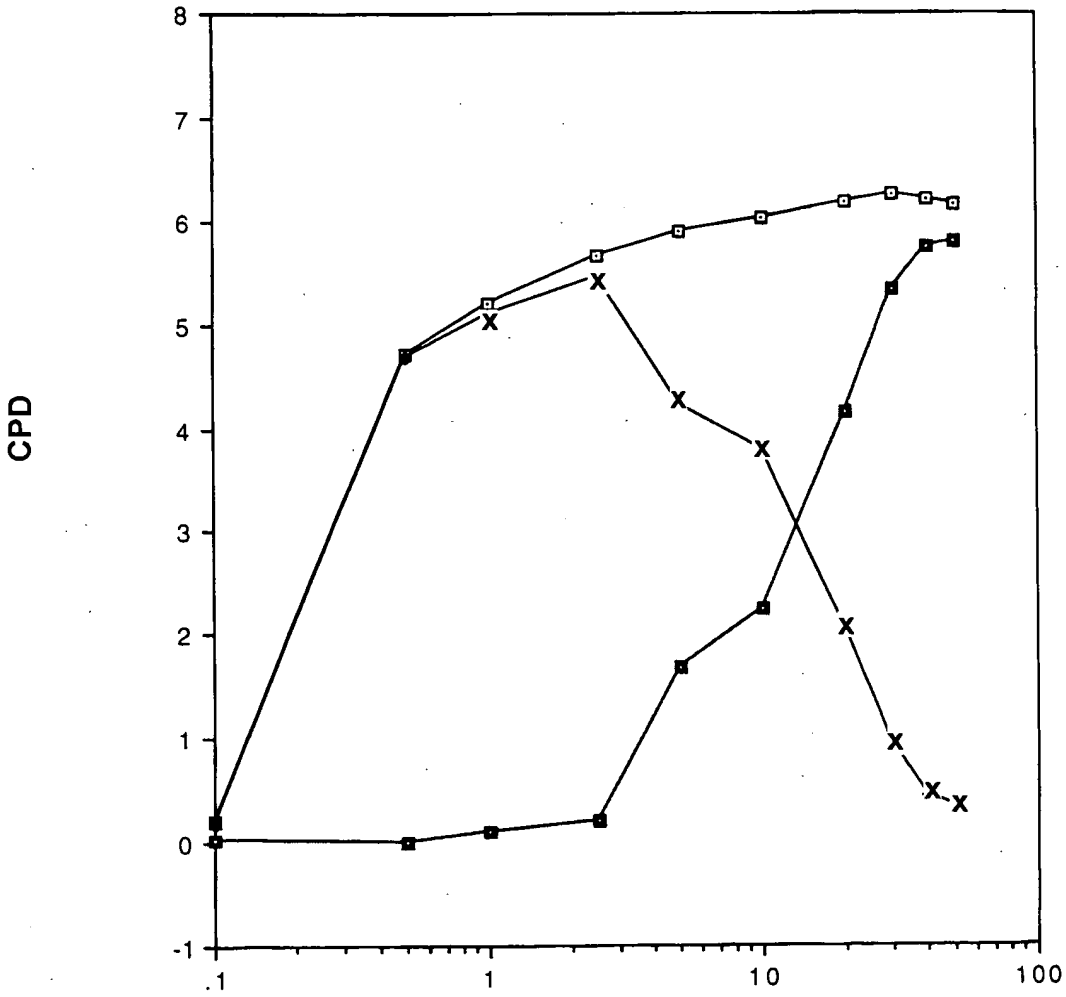
Open squares = + E_2

Closed squares = - E_2

FIGURE 28

HORSE SERUM TITRATION WITH T47D CELLS

EXTRACTION BY XAD-4 RESIN



LEGEND:

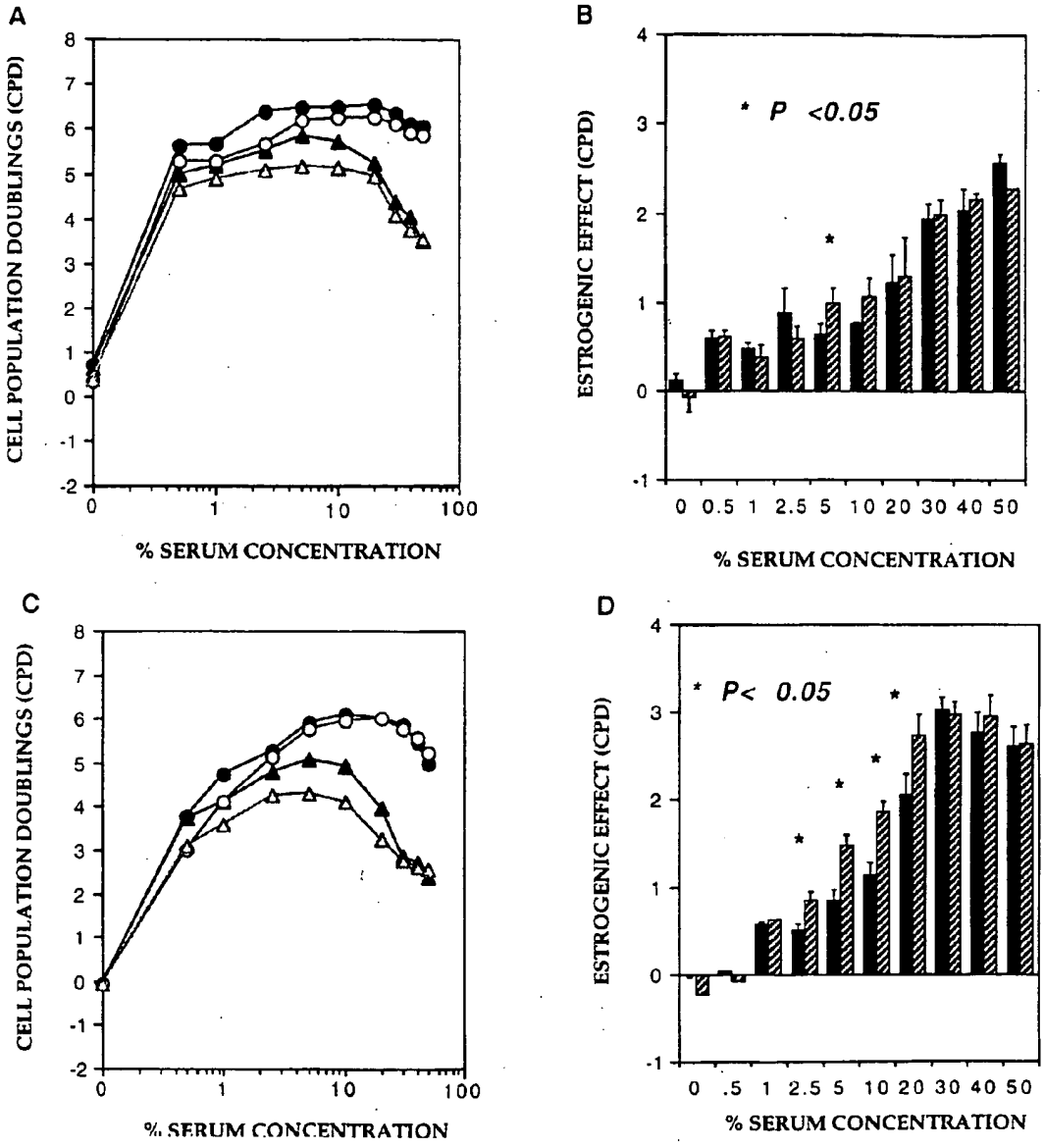
Open squares = + E_2

XXX = - E_2

Closed squares = Estrogenic effect

FIGURE 29

MCF-7 CELL GROWTH IN CDE - HORSE SERUM ± PHENOL RED AND ± E_2

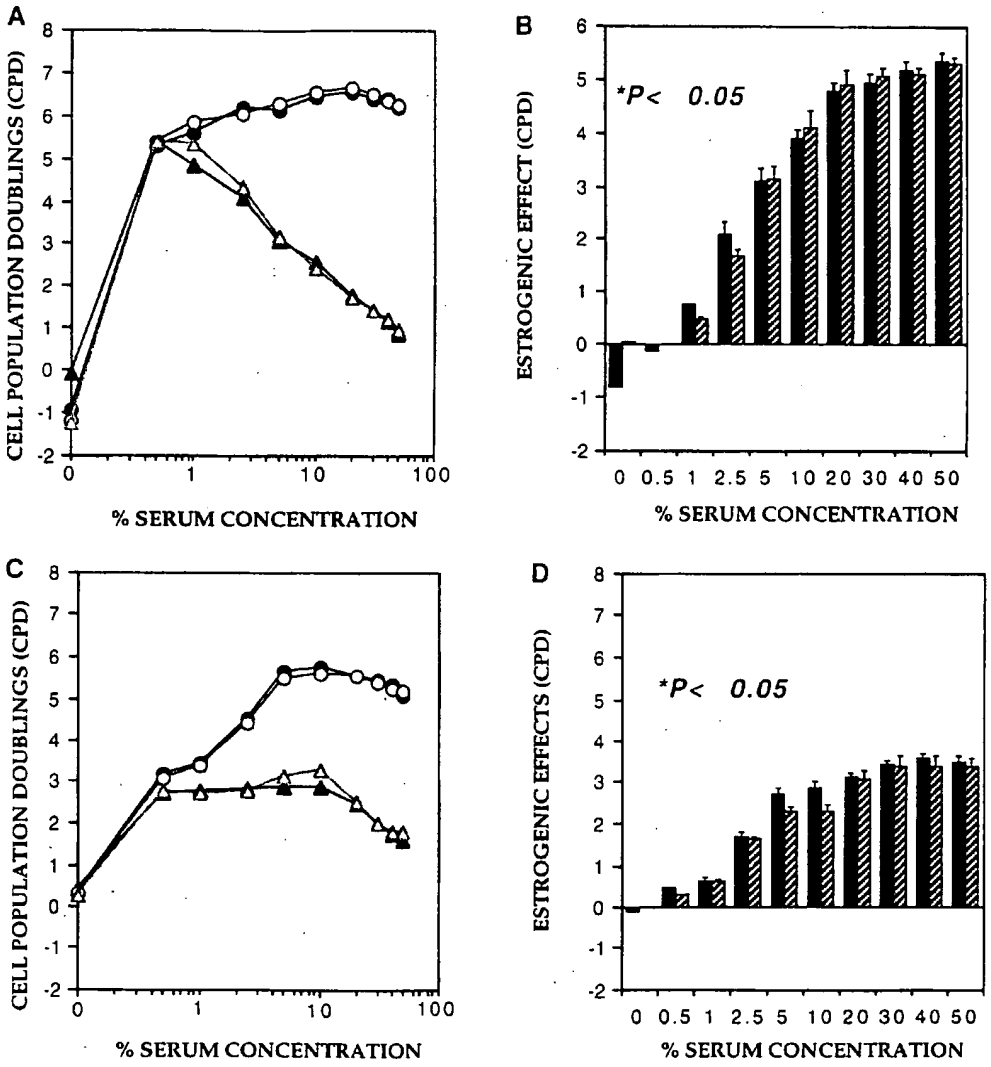


LEGEND:

(A) MCF-7A cell growth in phenol red containing medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).
(B) Estrogenic effects with MCF-7A cells in medium with phenol red (solid bars) and without phenol red (shaded bars) were calculated from (A) and defined as the CPD in medium containing E_2 minus the CPD in medium without added E_2 .
(C) MCF-7K cell growth in phenol red medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).
(D) Estrogenic effects with MCF-7K cells in medium with phenol red (solid bars) and without phenol red (shaded bars), calculated from (C).

FIGURE 30

T47D AND ZR-75-1 CELL GROWTH IN CDE-HS ± PHENOL RED AND ± E_2

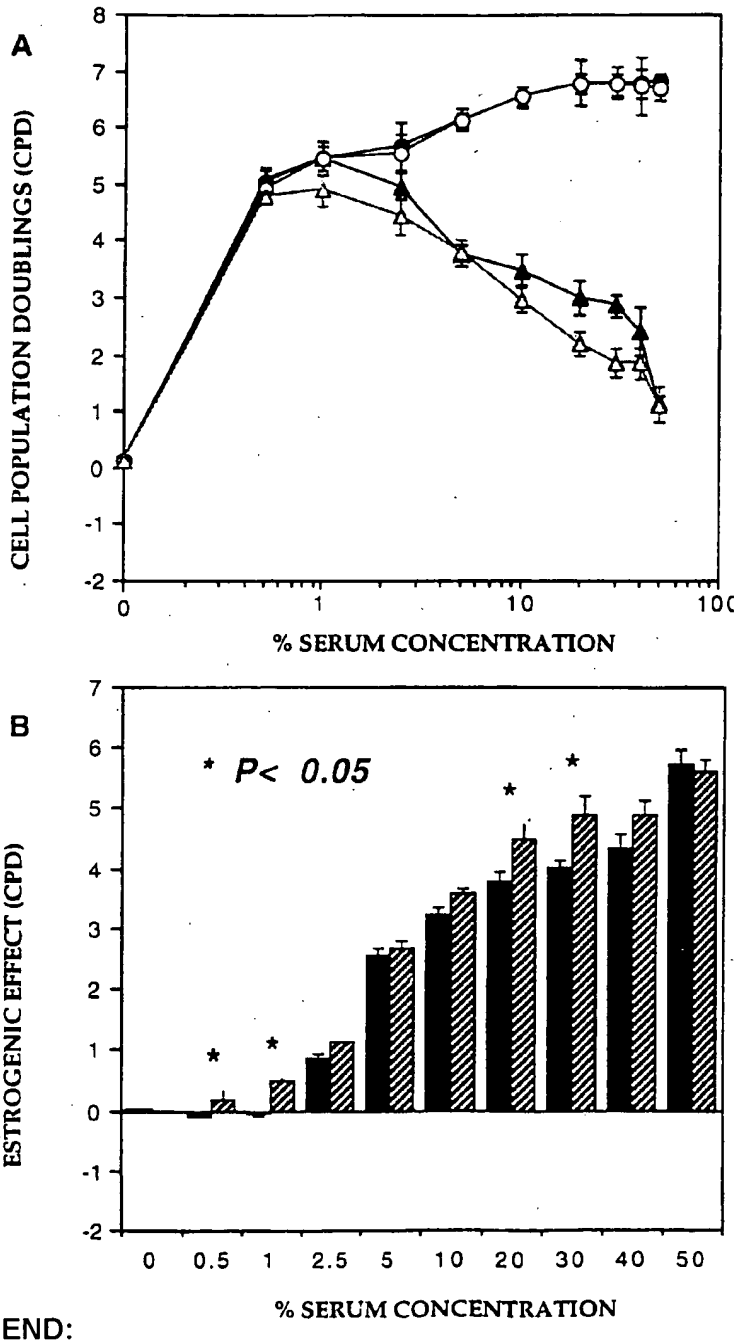


LEGEND:

(A) T47D cell growth in phenol red containing medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).
(B) Estrogenic effects with T47D cells in medium with phenol red (solid bars) and without phenol red (shaded bars) were calculated from (A) and defined as the CPD in medium containing E_2 minus the CPD in medium without added E_2.
(C) ZR-75-1 cell growth in phenol red medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).
(D) Estrogenic effects with ZR-75-1 cells in medium with phenol red (solid bars) and without phenol red (shaded bars), calculated from (C).

FIGURE 31

MTW9/PL2 CELL GROWTH IN CDE - HORSE SERUM ± PHENOL RED AND ± E_2



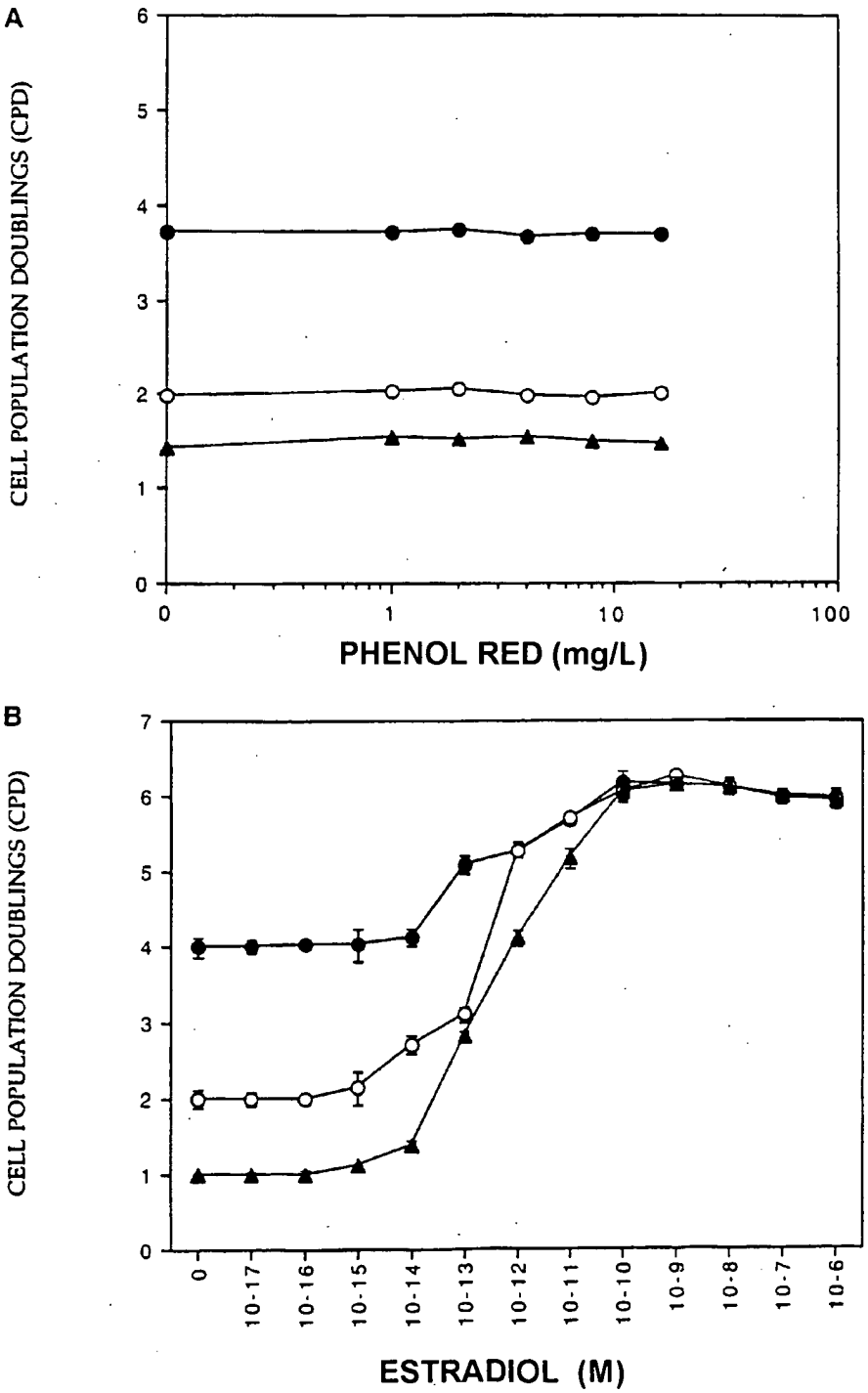
LEGEND:

(A) MTW9/PL2 growth in phenol red medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).

(B) Estrogenic effects with MTW9/PL2 cells in medium with phenol red (solid bars) and without (shaded bars) were calculated from (A).

FIGURE 32

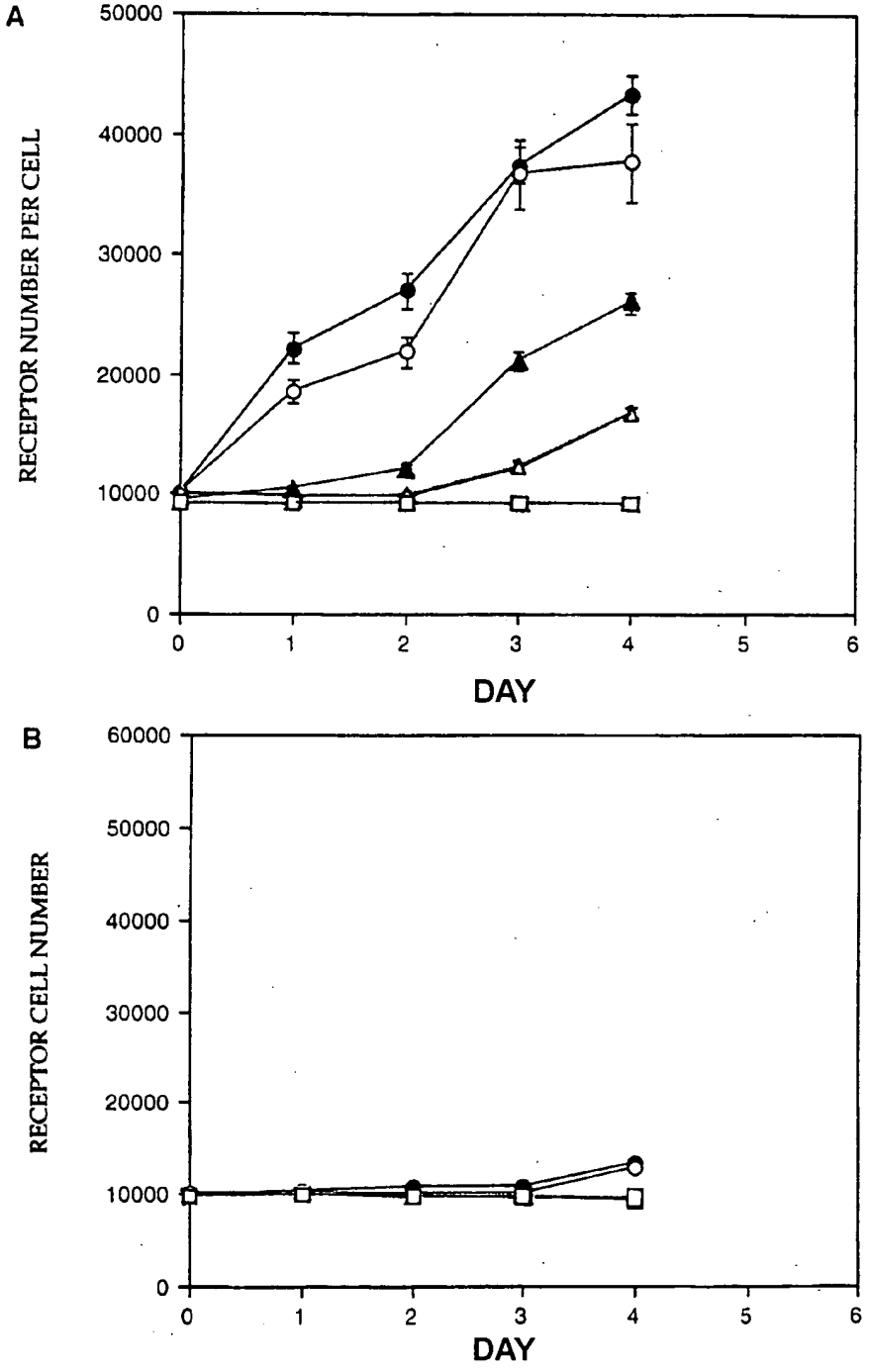
DOSE RESPONSE TO PHENOL RED AND E_2 IN THREE CELL LINES



LEGEND: The growth of the MCF-7A (closed circles), MTW9/PL2 (open circles) and T47D (closed triangles) cell lines was assessed at 14, 7, and 12 days.

FIGURE 33

PROGESTERONE RECEPTOR INDUCTION IN T47D CELLS BY PHENOL RED AND E_2



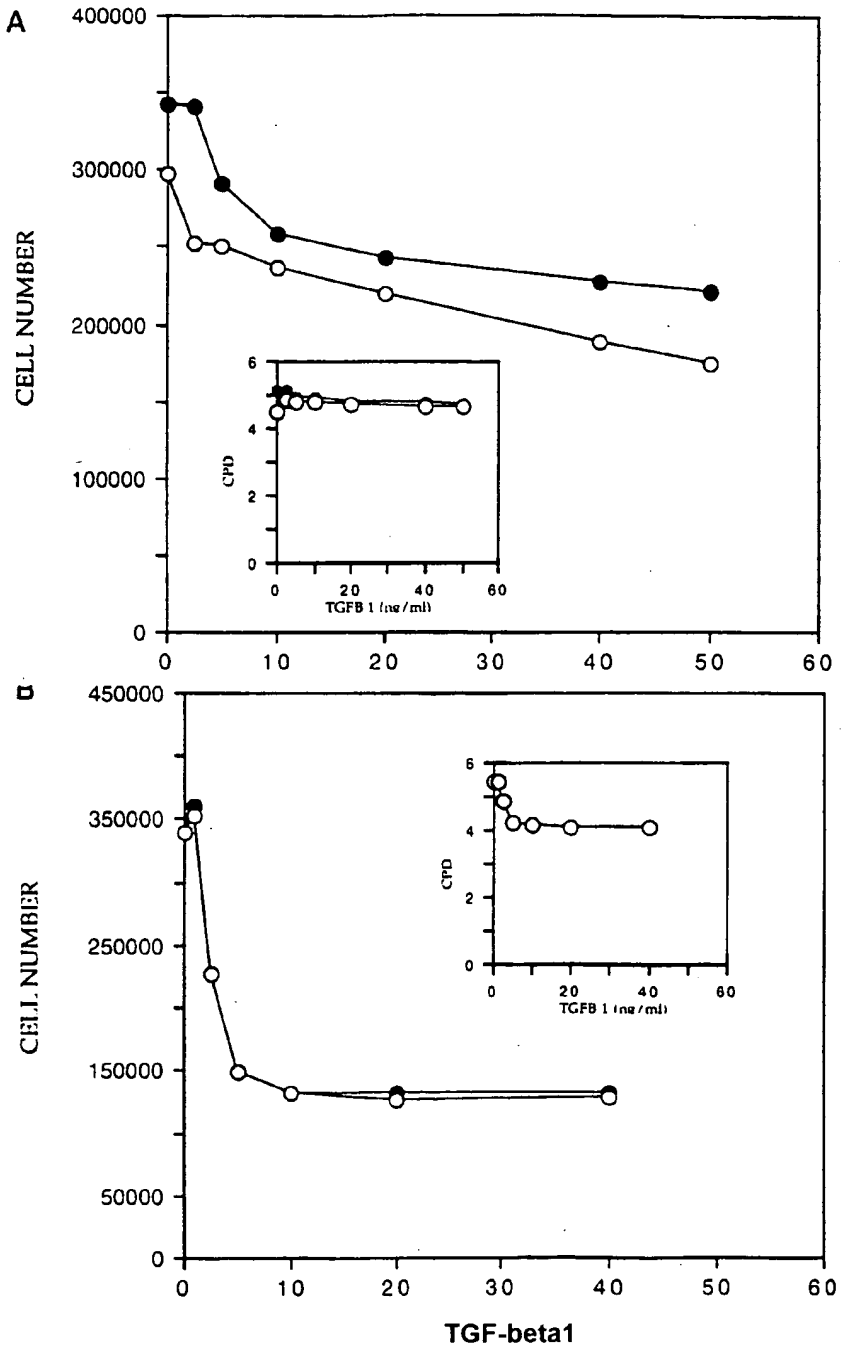
LEGEND:

(A) The effects of E_2 at 1.0×10^{-8} M (closed circles), 1.0×10^{-10} M (open circles), 1.0×10^{-12}M (closed triangles), 1.0×10^{-14} M (open triangles) and the control without added E_2 (open squares).

(B) The effects of phenol red at 16 mg/L (closed circles), 8mg/L (open circles), 4 mg/L (closed triangles), 2 mg/L (open triangles), and the control without phenol red (open squares).

FIGURE 34

EFFECT OF TGF-beta1 ON THE GROWTH OF BREAST/MAMMARY ORIGIN CELL LINES



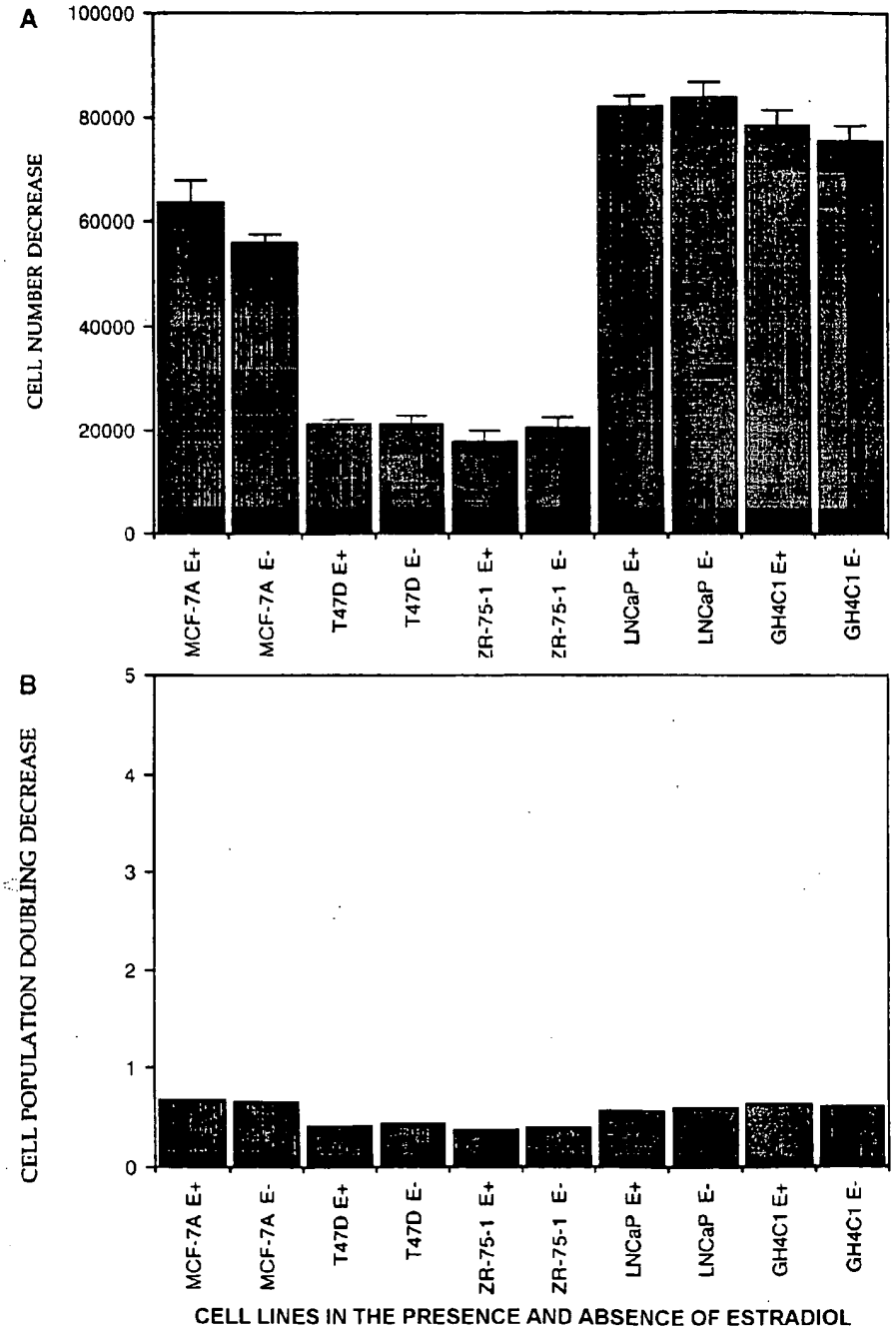
LEGEND:

(A) The effect of the transforming growth inhibitor on human breast MCF-7K cell growth as measured after 12 d either with 10 nM E_2 (closed circles) or without the hormone (open circles). The insert shows conversion of the cell number results to CPD.

(B) The same experiment with rat mammary MTW9/PL2 cells after 9 d growth.

FIGURE 35

EFFECT OF TGF-beta1 ON THE GROWTH OF CELL LINES FROM BOTH HUMAN AND RODENT TUMORS

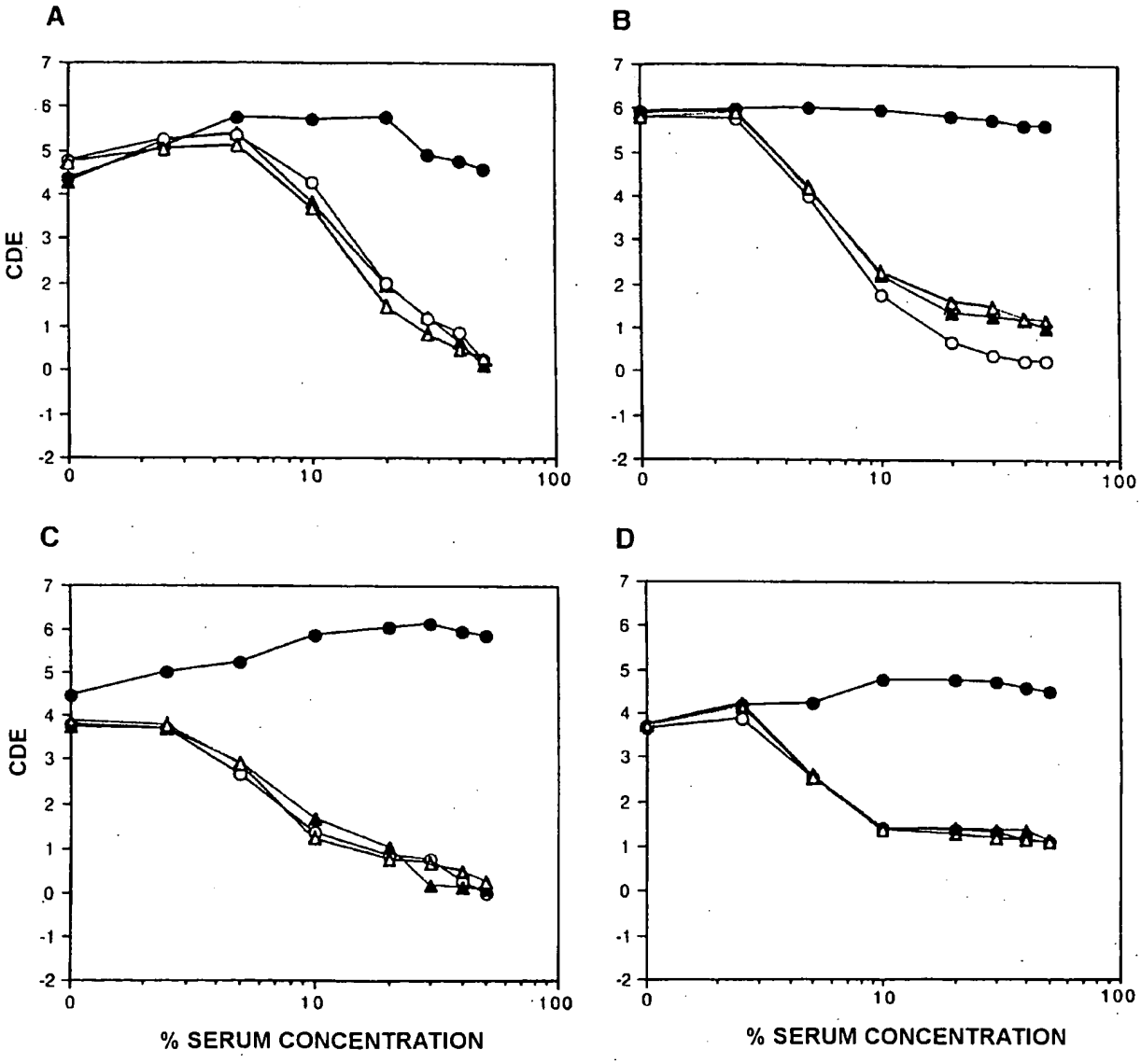


In these studies, TGF-beta1 was added at 40 ng/ml. Estradiol ($\pm$ E) indicates either no added E_2 or the steroid at 10 nM.

(A) The effect of TGF-beta1 on five cell lines after 10-14 d growth in medium $\pm$ E_2. The results are expressed as cell number decreases caused by TGF-beta1.
(B) The CPD decreases caused by TGF-beta1 $\pm$ E_2 with each of the cell lines shown in (A).

FIGURE 36

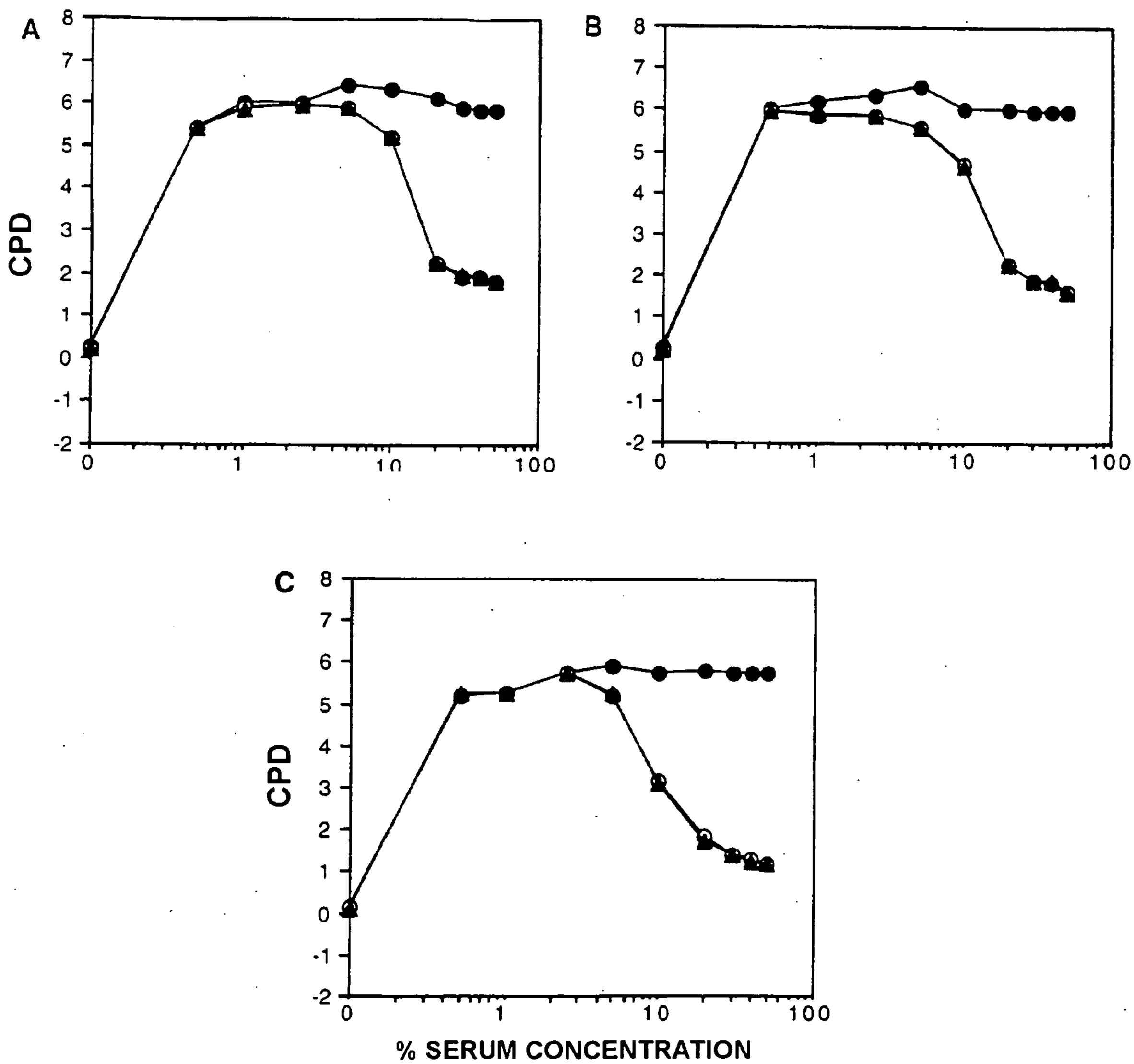
EFFECT OF EGF AND TGF-alpha ON THE GROWTH OF HUMAN BREAST CANCER CELLS



The cells were grown in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum. Each line tested was grown in serum alone (open circles) and in serum plus 50 ng/ml EGF (open triangles), 50 ng/ml TGF-alpha (closed triangles), or 10 nM E_2 without exogenous growth factors (closed circles). (A) - (D) show the results with the MCF-7A, MCF-7K, T47D, and ZR-75-1 cell lines, respectively.

FIGURE 37

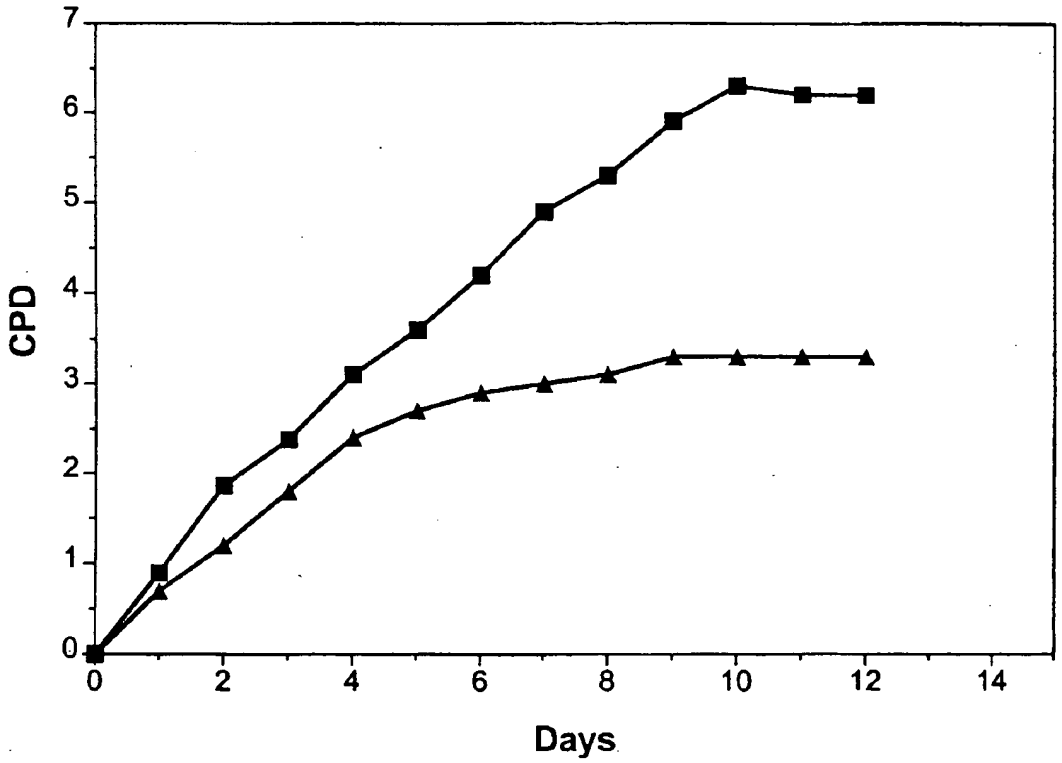
EFFECT OF IGF-I ON THE GROWTH OF HUMAN BREAST CANCER CELLS



Breast cancer cells were grown in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum. Each cell line tested was grown in serum alone (open circles) and in serum plus 1.0 ug/ml IGF-I (triangles), or in serum with 10 nM E_2 without exogenous growth factors (closed circles). (A) - (C) show the results with the MCF-7K, MCF-7A and T47D cells, respectively. Assays were conducted for 12-14 d.

FIGURE 38

T47D CELLS IN STANDARD D-MEM/F-12 MEDIUM VS "LOW FE" SERUM-FREE SERUM



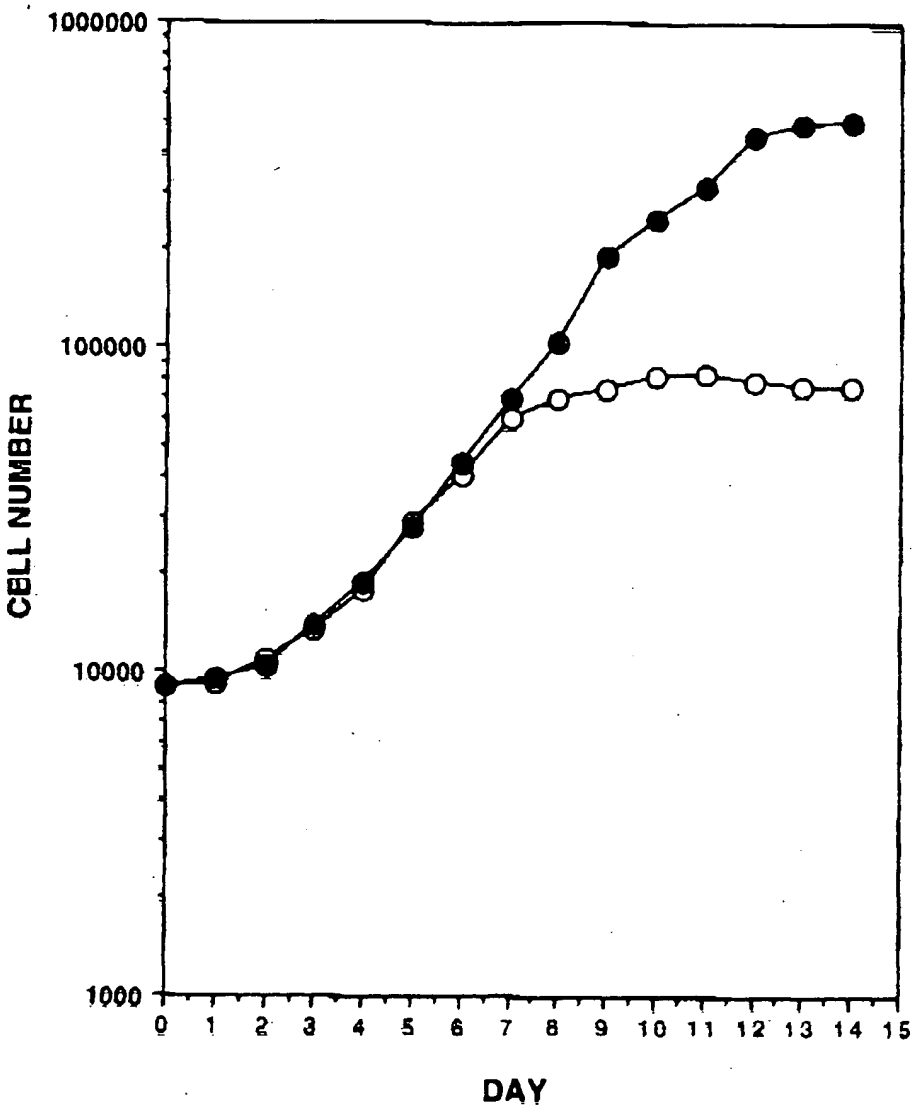
LEGEND:

"STANDARD" MEDIUM

"LOW-FE" MEDIUM

FIGURE 39

LNCaP CELLS IN STANDARD D-MEM/F-12 MEDIUM
VS "LOW-FE" SERUM-FREE MEDIUM



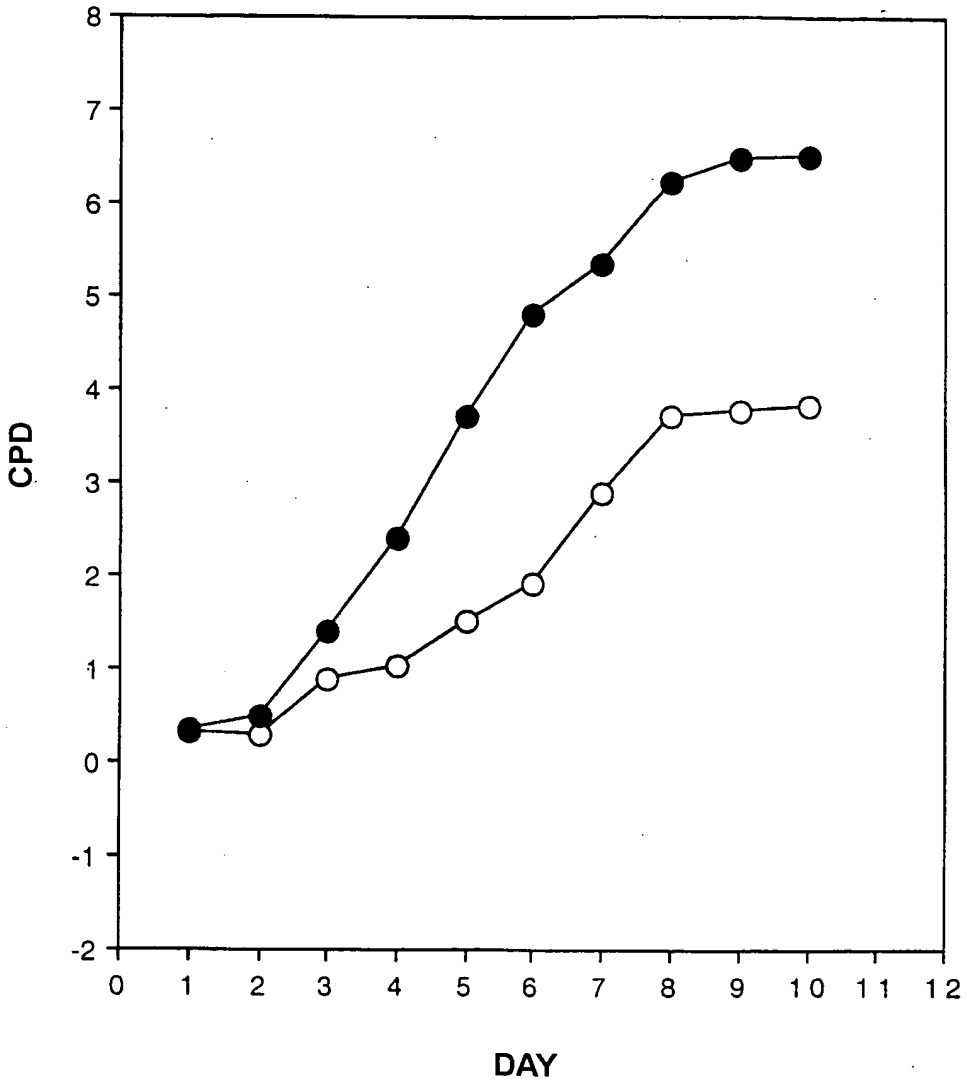
LEGEND:

"STANDARD" MEDIUM

"LOW-FE" MEDIUM

FIGURE 40

MDCK CELLS IN STANDARD D-MEM/F-12 MEDIUM
VS "LOW FE" SERUM-FREE MEDIUM



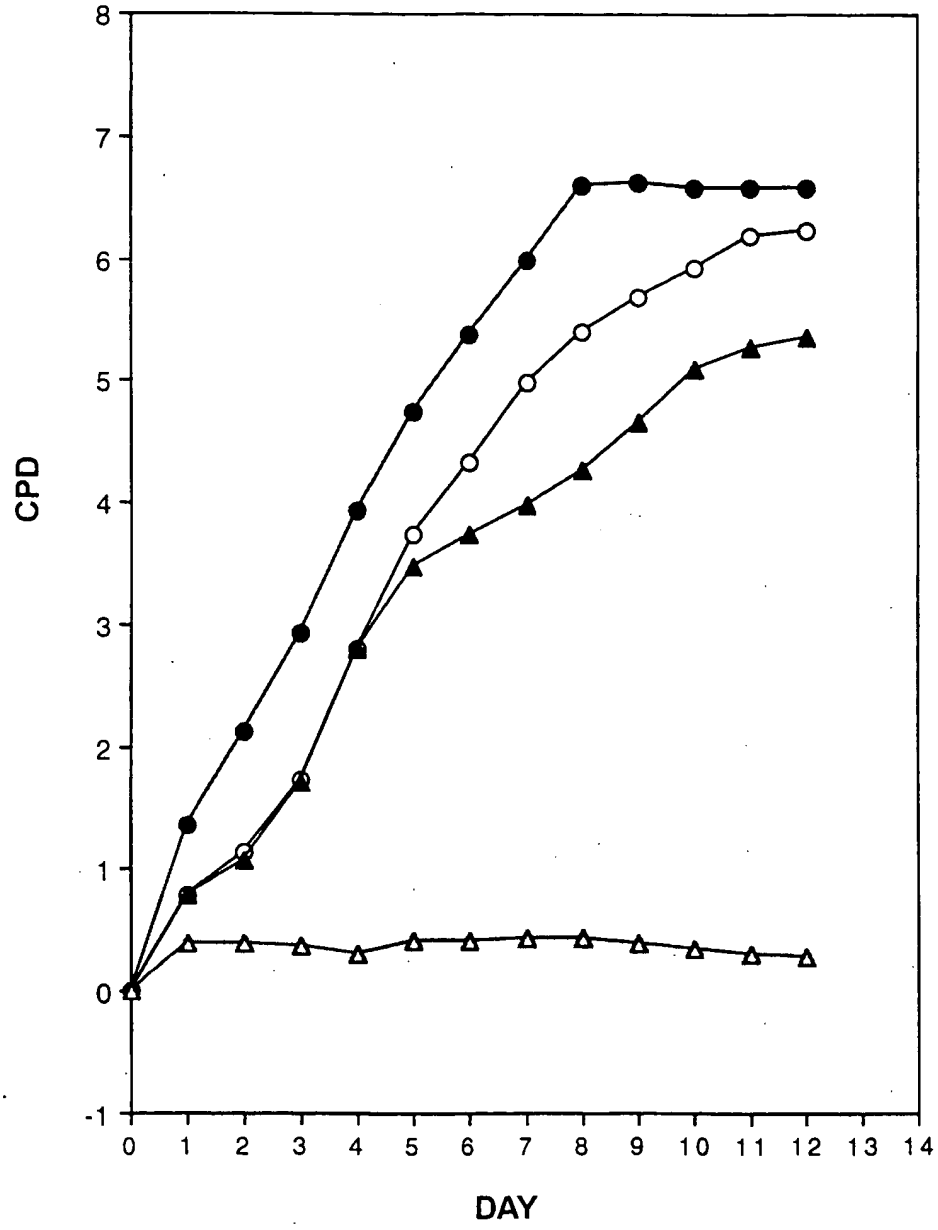
LEGEND:

"STANDARD" MEDIUM

"LOW-FE" MEDIUM

FIGURE 41

LNCaP CELL GROWTH IN CAPM ± DHT AND 10% FETAL BOVINE SERUM

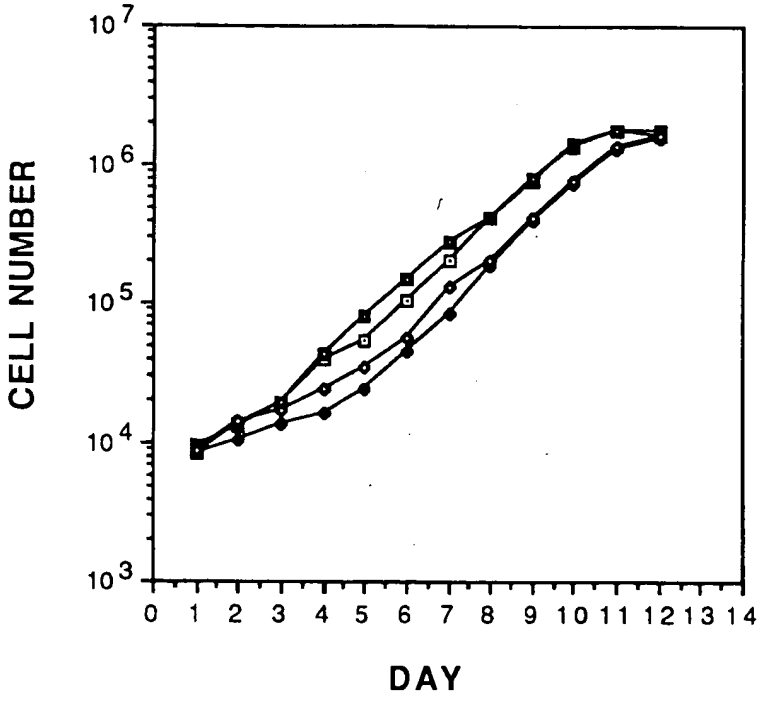


LEGEND:

Closed circles = Fetal bovine serum
Open circles = CAPM + DHT
Closed triangles = CAPM - DHT
Open triangles = D-MEM/F12 only

FIGURE 42

PC3 AND DU145 GROWTH IN SERUM - FREE MEDIUM VS MEDIUM WITH 10% FETAL CALF SERUM



LEGEND:

PC3 IN SERUM-FREE MEDIUM

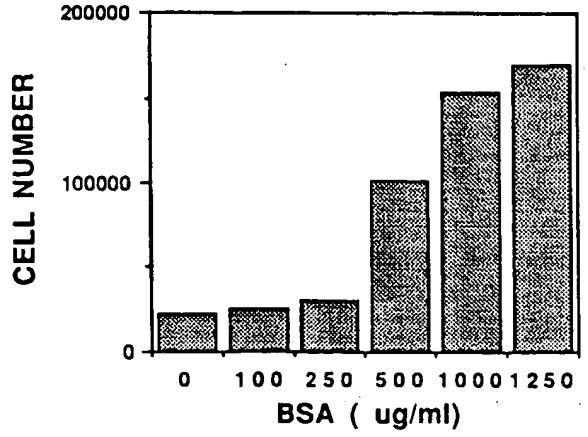
DU145 IN SERUM-FREE MEDIUM

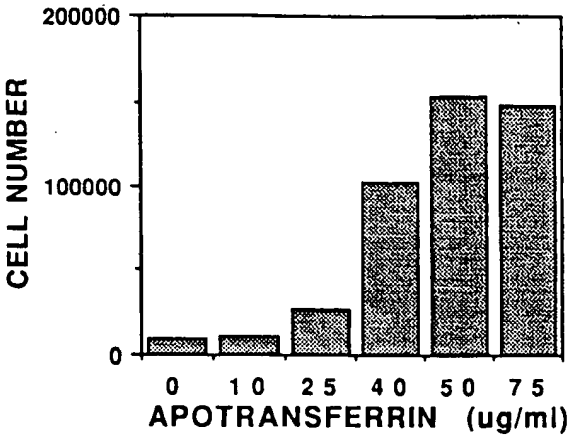
PC3 IN 10% FETAL CALF SERUM

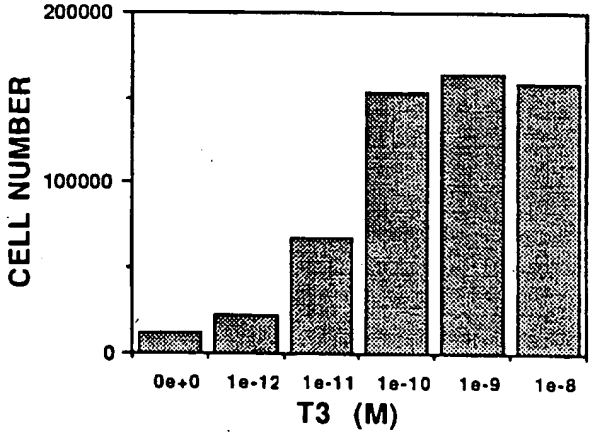
DU145 IN 10% FETAL CALF SERUM

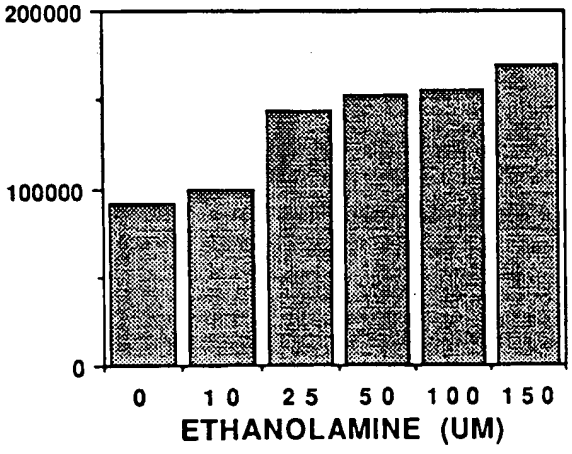
FIGURE 43

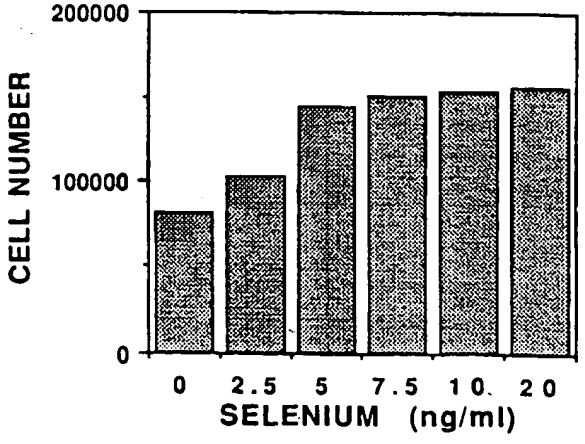
DOSE RESPONSE EFFECTS OF CAPM SERUM - FREE MEDIUM COMPONENTS











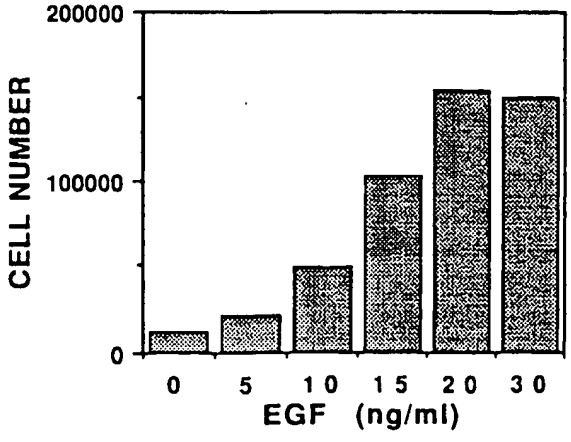
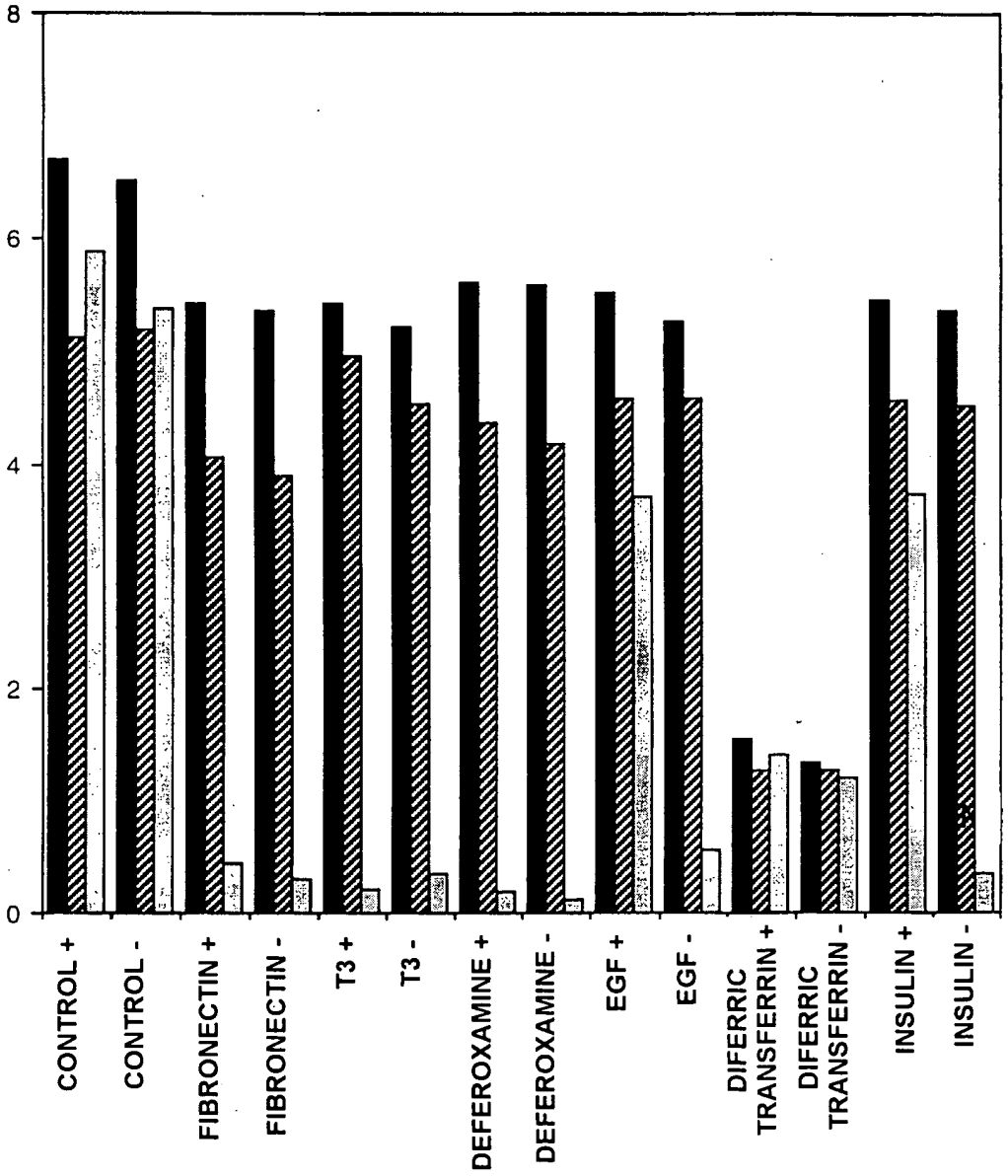


FIGURE 44

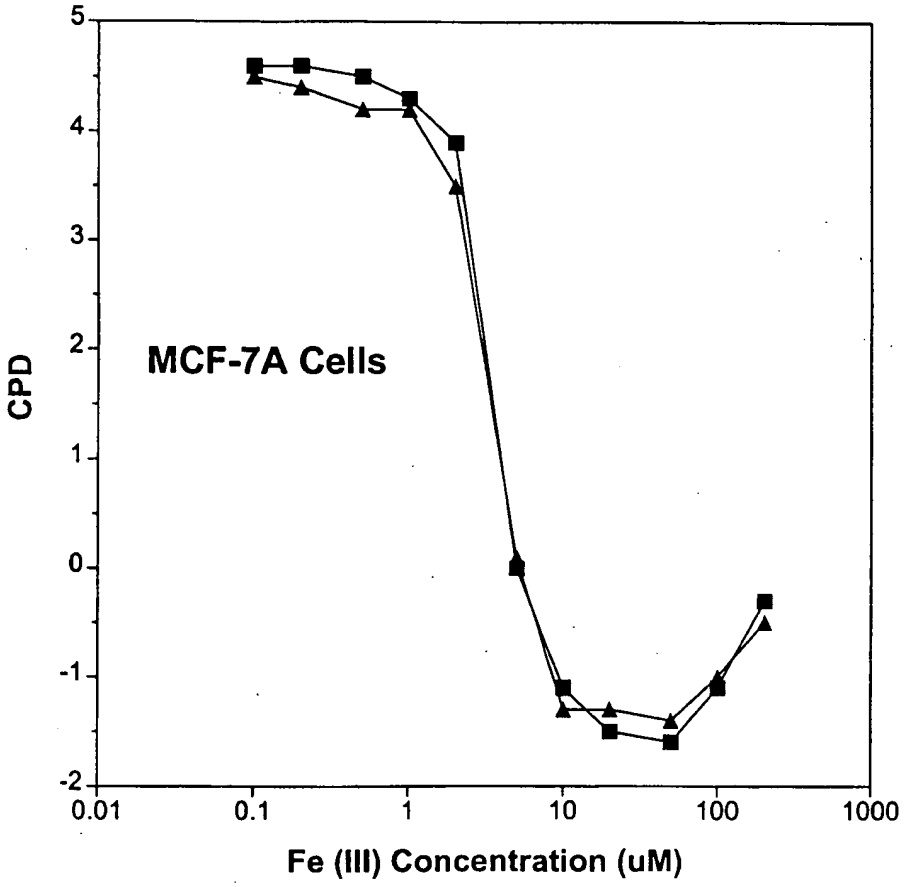
DELETIONS OF INDIVIDUAL COMPONENTS OF CAPM WITH PROSTATE CANCER CELL LINES



LEGEND:
- ■ = PC3
- ▨ = DU145
- ▫ = LNCaP
- \+ = 10 nM DHT
- − = NO DHT

FIGURE 45

EFFECT OF FE (III) IN MCF-7A CELL GROWTH IN DDM-2MF DEFINED MEDIUM



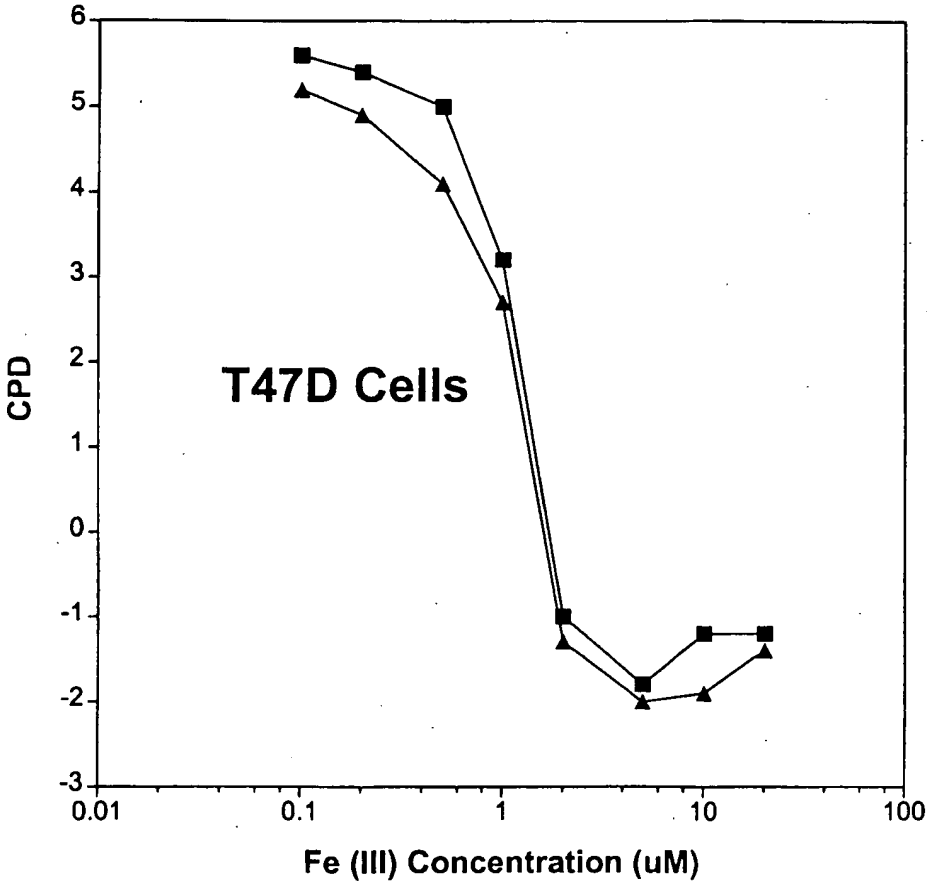
LEGEND:

plus E_2

minus E_2

FIGURE 46

EFFECT OF FE (III) IN T47D CELL GROWTH IN DDM-2MF DEFINED MEDIUM



LEGEND:

plus E_2

minus E_2

FIGURE 47

EFFECTS OF INCREASING CONCENTRATIONS OF IRON ON LNCaP CELLS GROWN IN SERUM-FREE MEDIUM WITH APOTRANSFERRIN

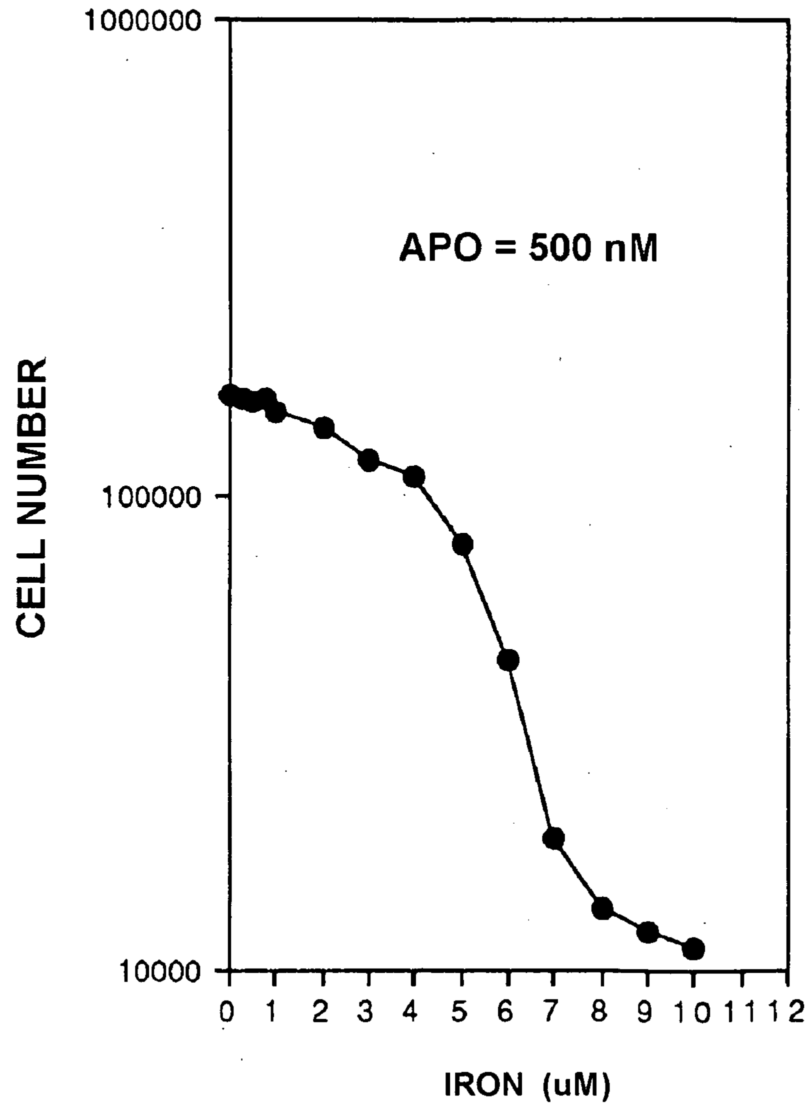
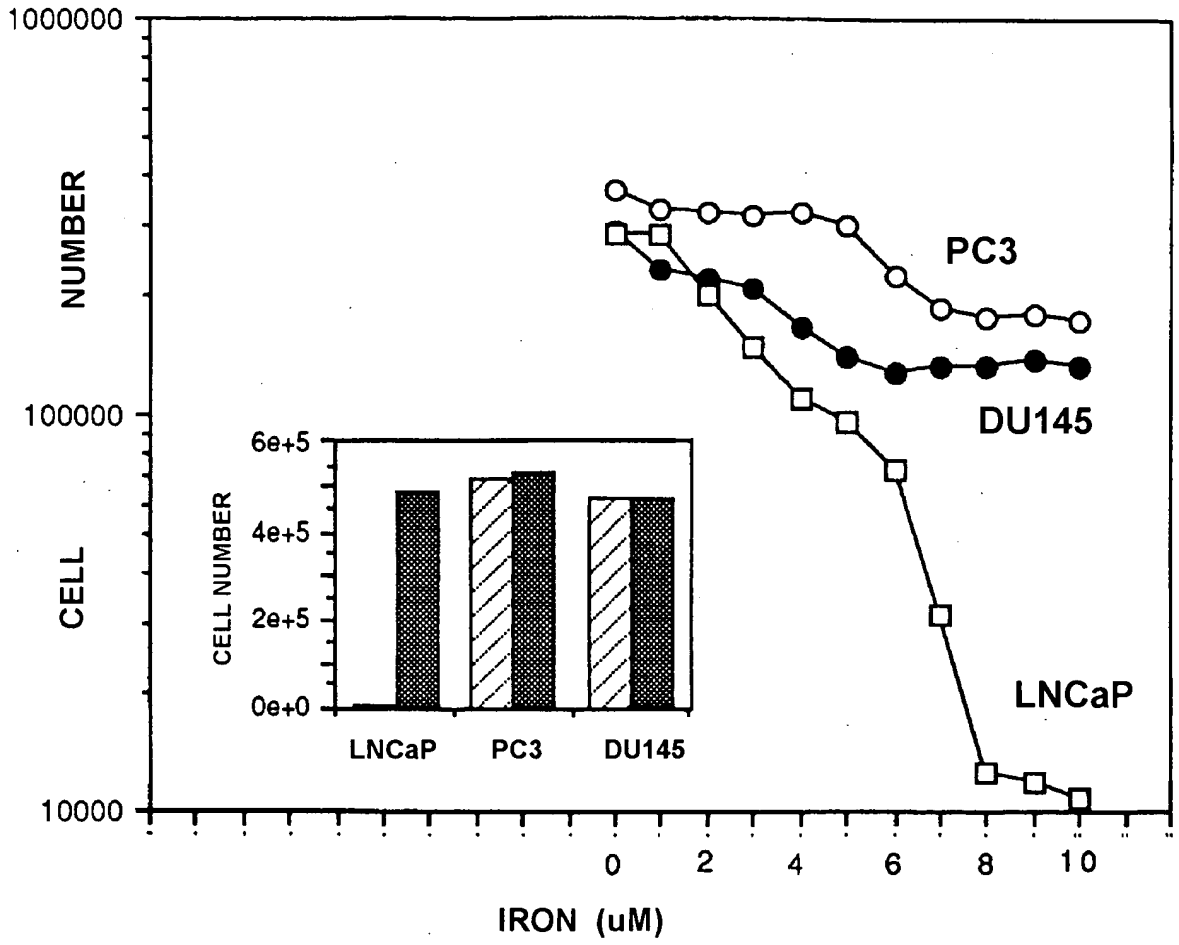


FIGURE 48

EFFECTS OF IRON AND T_3 ON THREE PROSTATIC CELL LINES IN SERUM-FREE MEDIUM



INSERT:

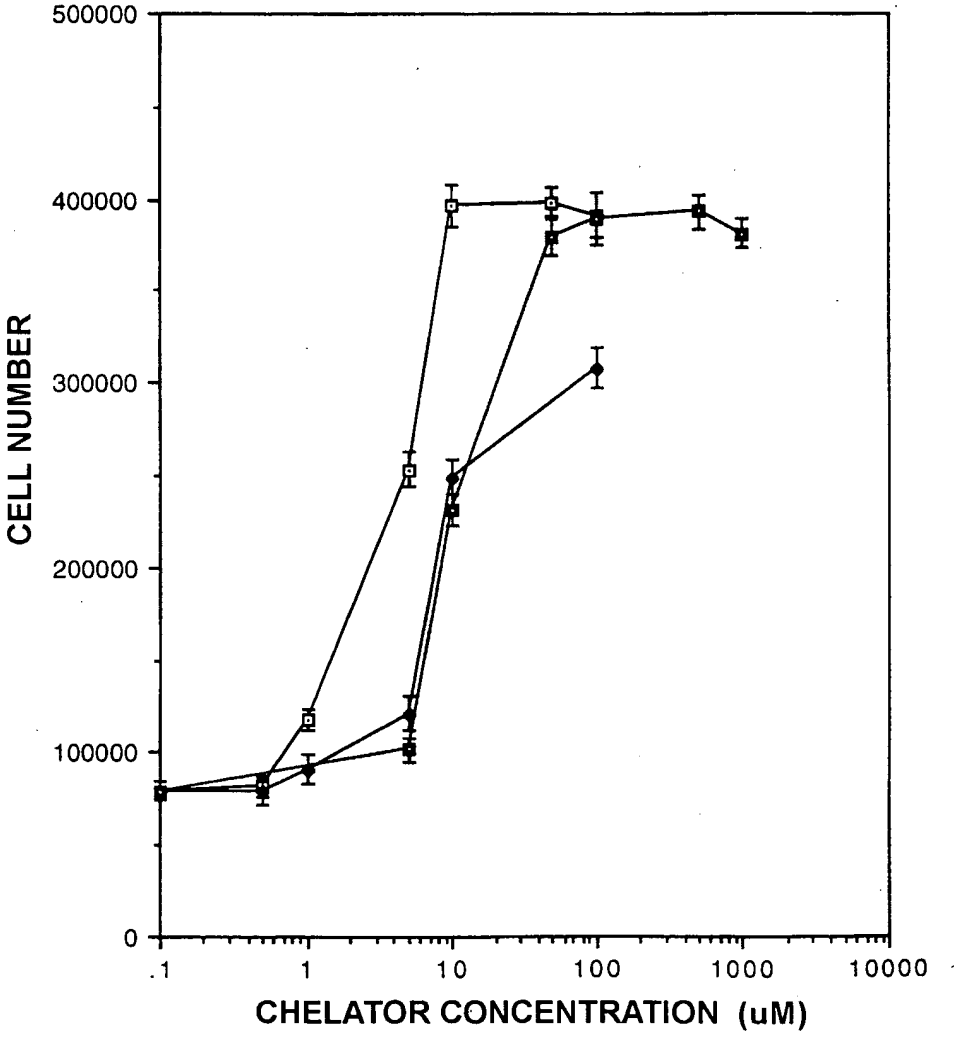
DARK BARS = GROWTH IN CAPM PLUS T_3

LIGHT (HATCHED) BARS = GROWTH IN CAPM MINUS T_3

NOTE THE STRIKING DEPENDENCE OF LNCaP CELLS ON T_3

FIGURE 49

EFFECT OF CHELATORS ON SERUM-FREE T47D GROWTH UNDER HIGH IRON CONDITIONS

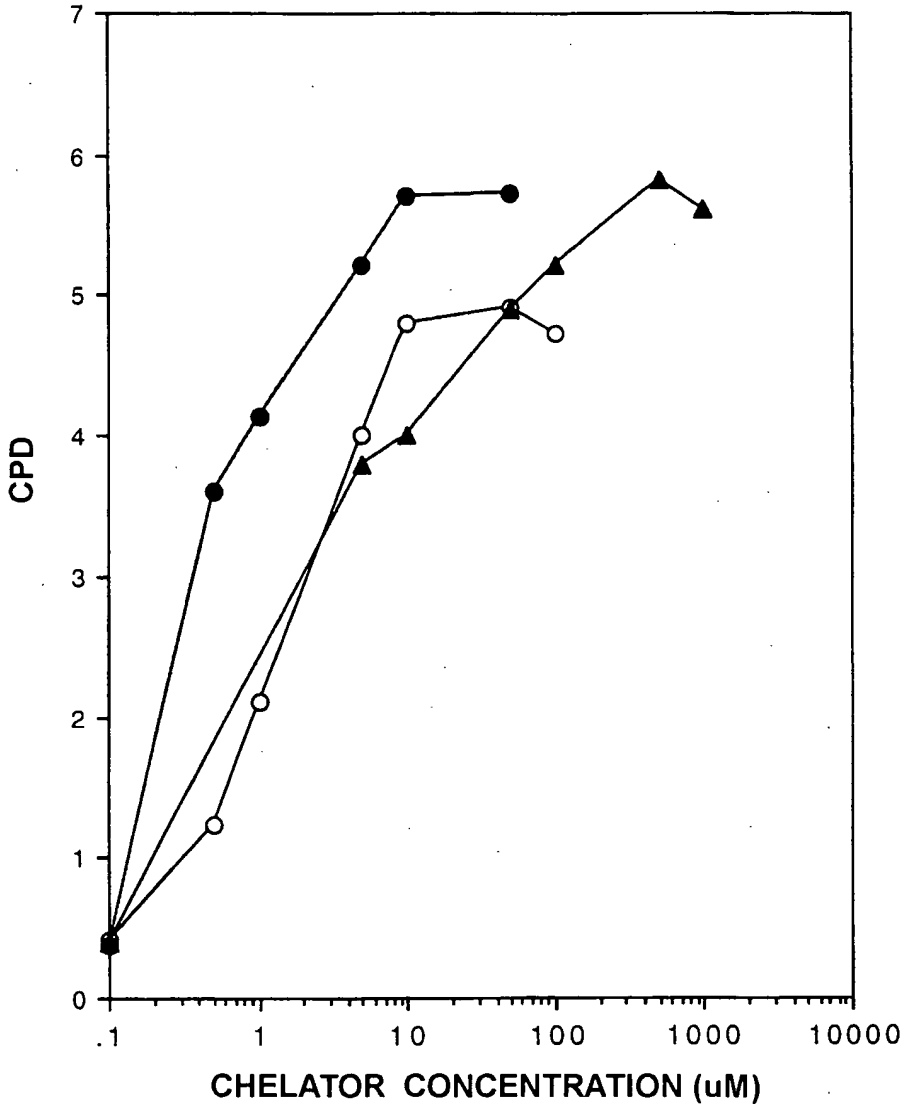


LEGEND:

- DEFEROXAMINE
- EDTA
- CITRATE

FIGURE 50

EFFECT OF CHELATORS ON SERUM-FREE LNCaP GROWTH UNDER HIGH IRON CONDITIONS



LEGEND:

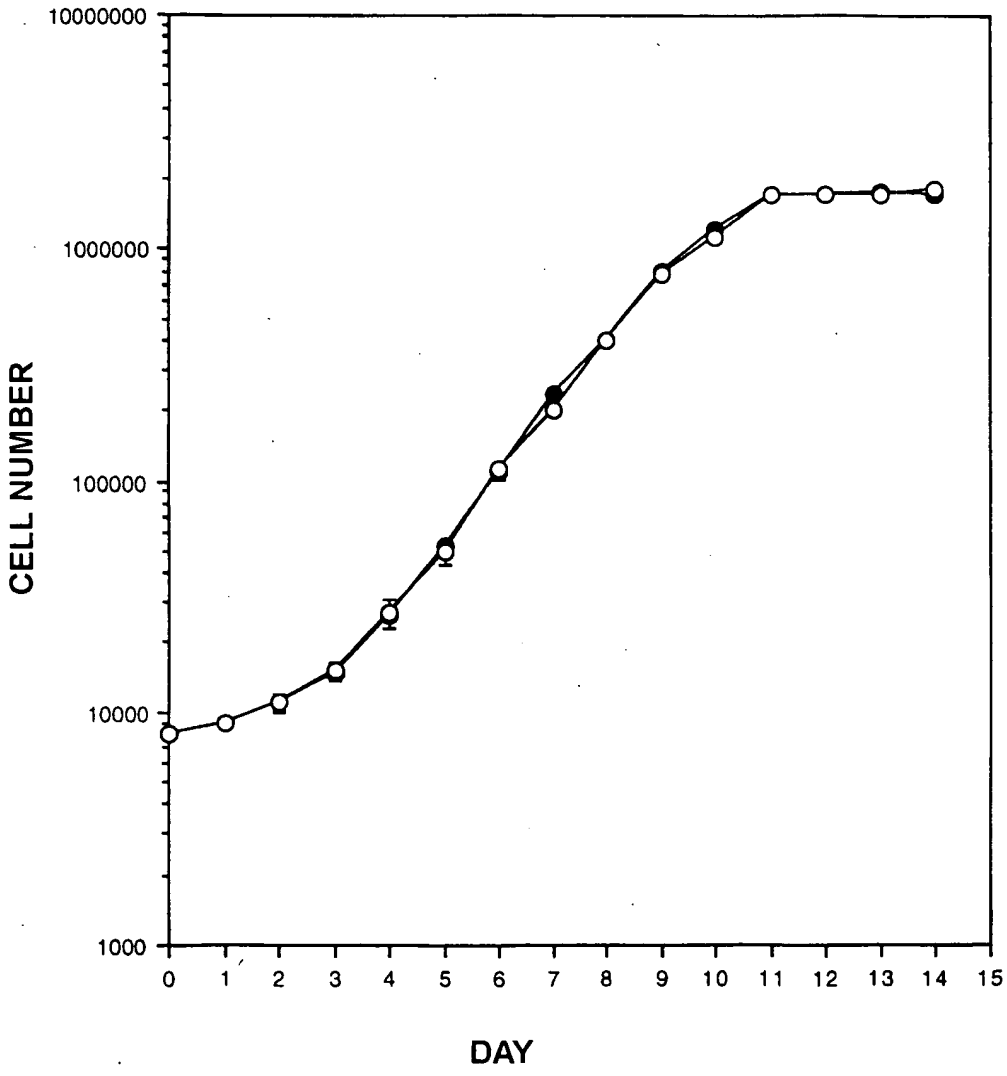
Closed circles = Deferoxamine

Open circles = Citrate

Closed triangles = EDTA

FIGURE 51

DU145 GROWTH IN SERUM-FREE MEDIUM BASED ON "LOW FE" OR "STANDARD" MEDIUM



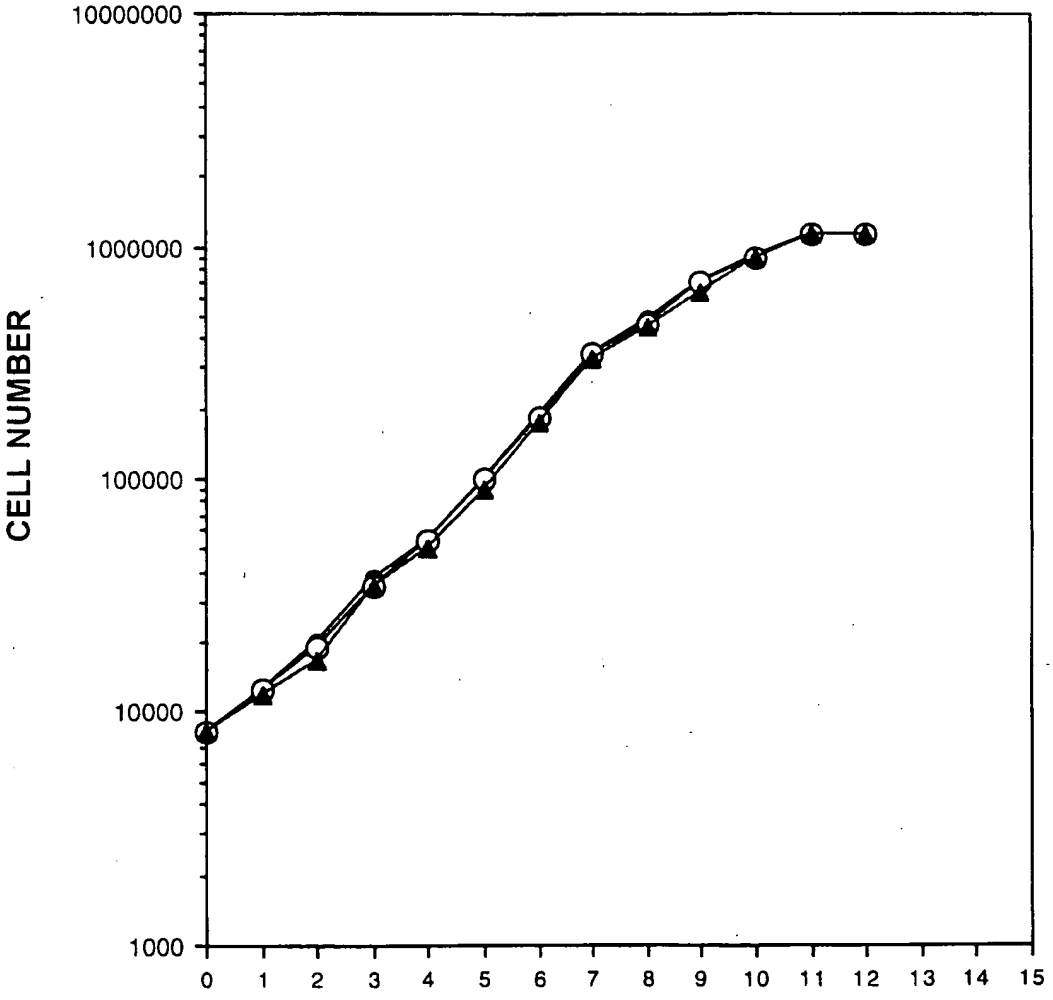
LEGEND:

Open circles = "Low Fe" medium

Closed circles = "Standard" medium

FIGURE 52

PC3 GROWTH IN SERUM-FREE MEDIUM
BASED ON "LOW FE" OR "STANDARD" MEDIUM



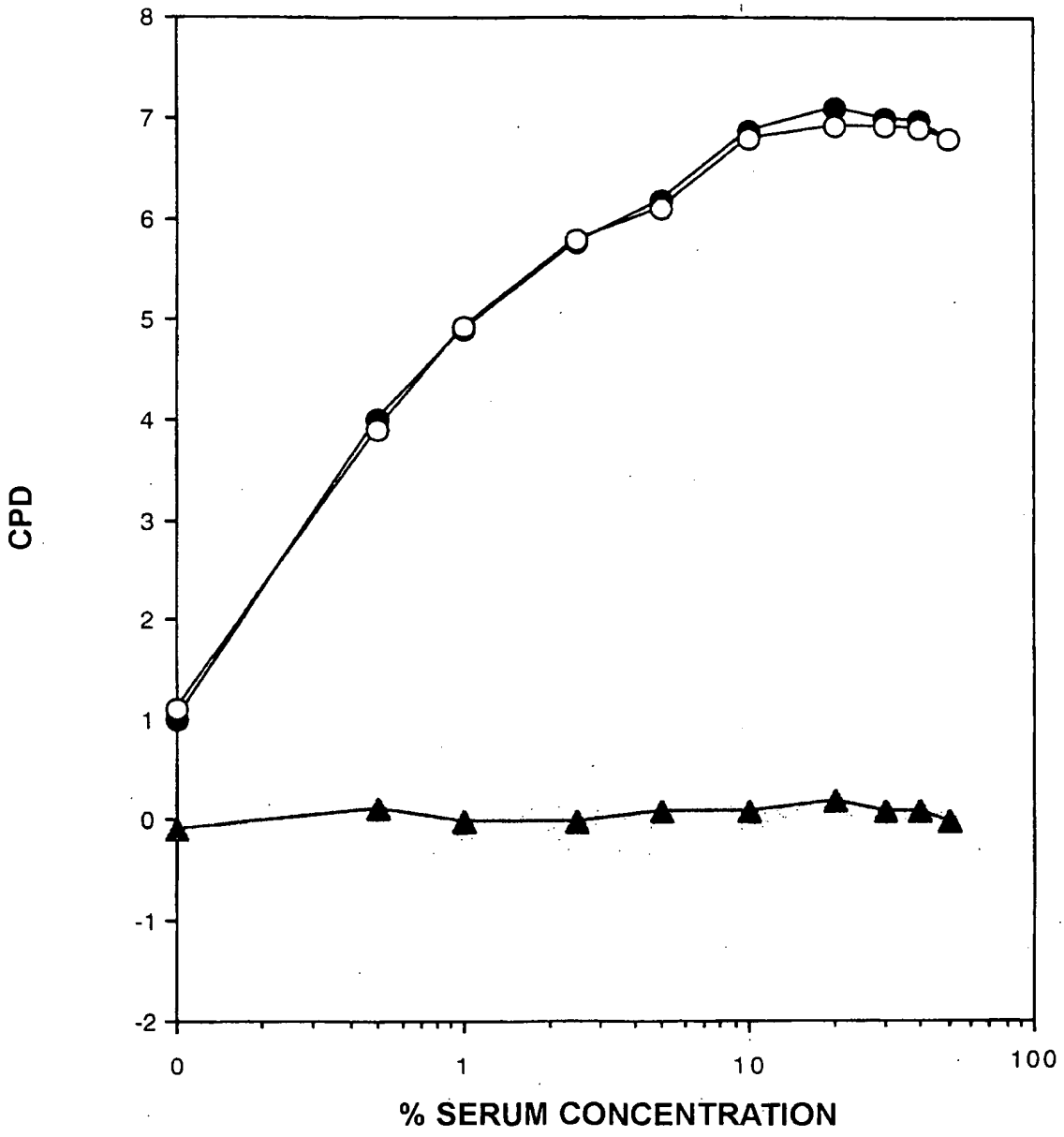
LEGEND:

Open circles = "Low Fe" medium

Closed triangles = "Standard" medium

FIGURE 53

CDE HORSE SERUM TITRATION ON DU145 CELLS



LEGEND:

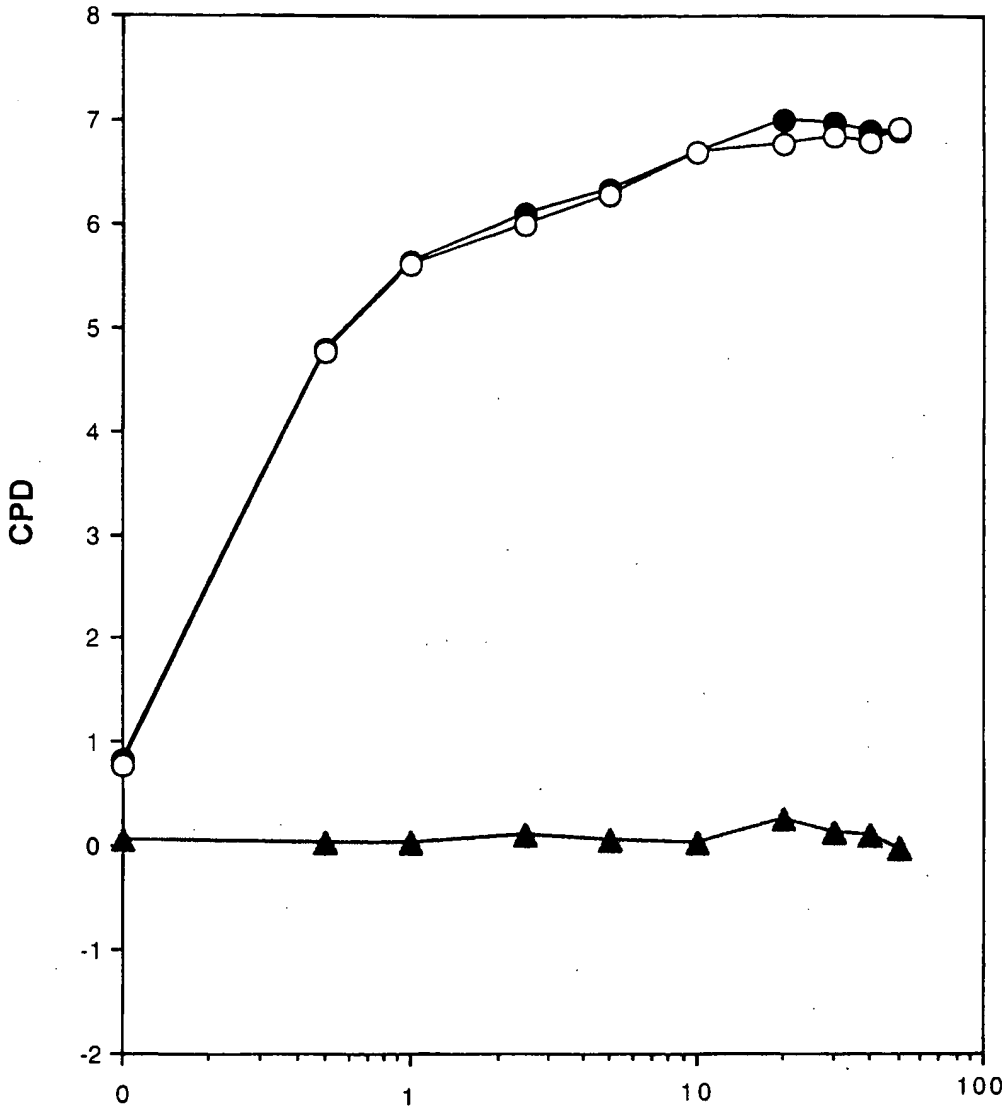
= + 10 nM DHT

= STEROID FREE

= ANDROGENIC EFFECT

FIGURE 54

CDE HORSE SERUM TITRATION ON PC3 CELLS



LEGEND:

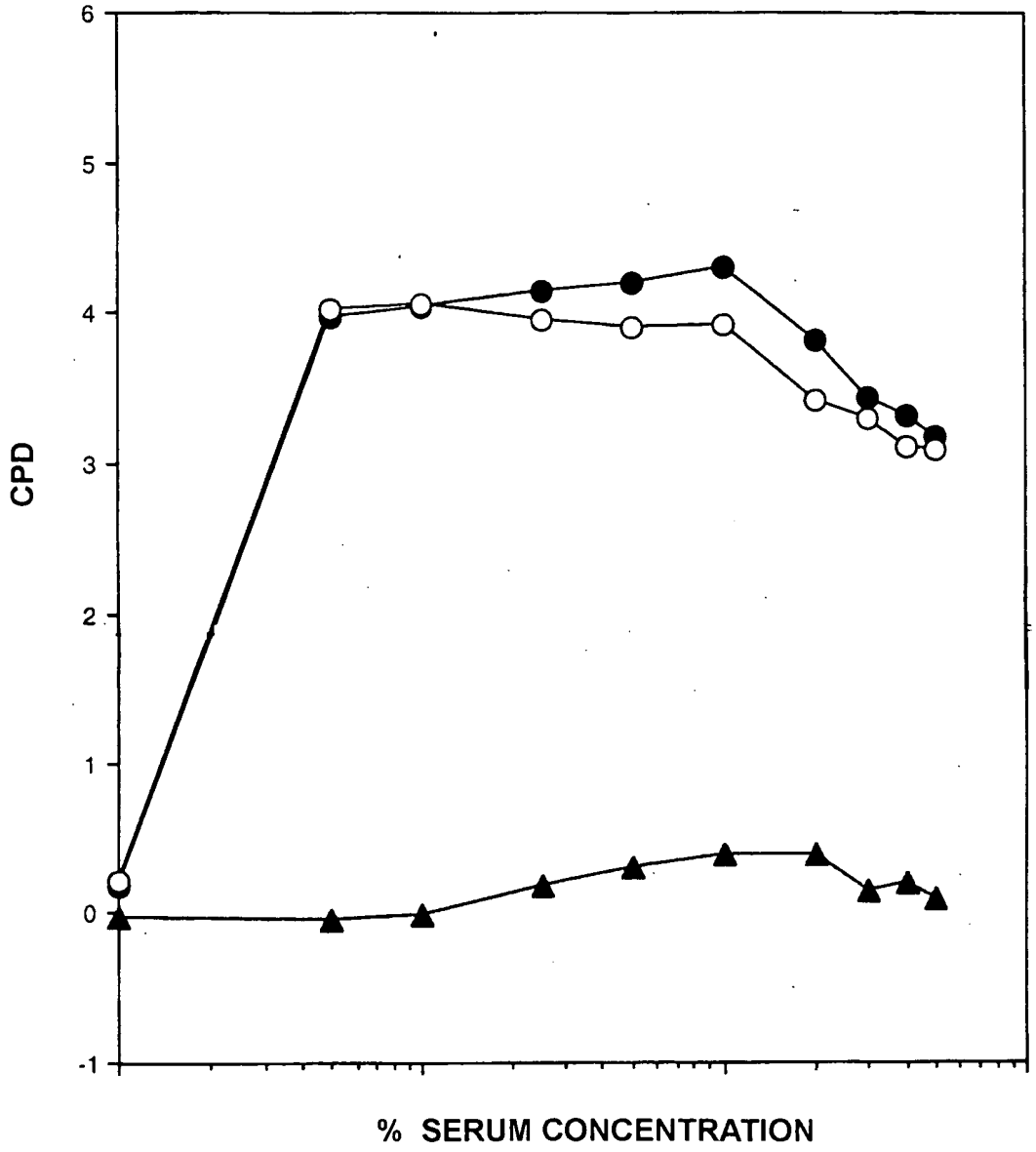
= + 10 nM DHT

= STEROID FREE

= ANDROGENIC EFFECT

FIGURE 55

CDE HORSE SERUM TITRATION ON ALVA-41 CELLS



LEGEND:

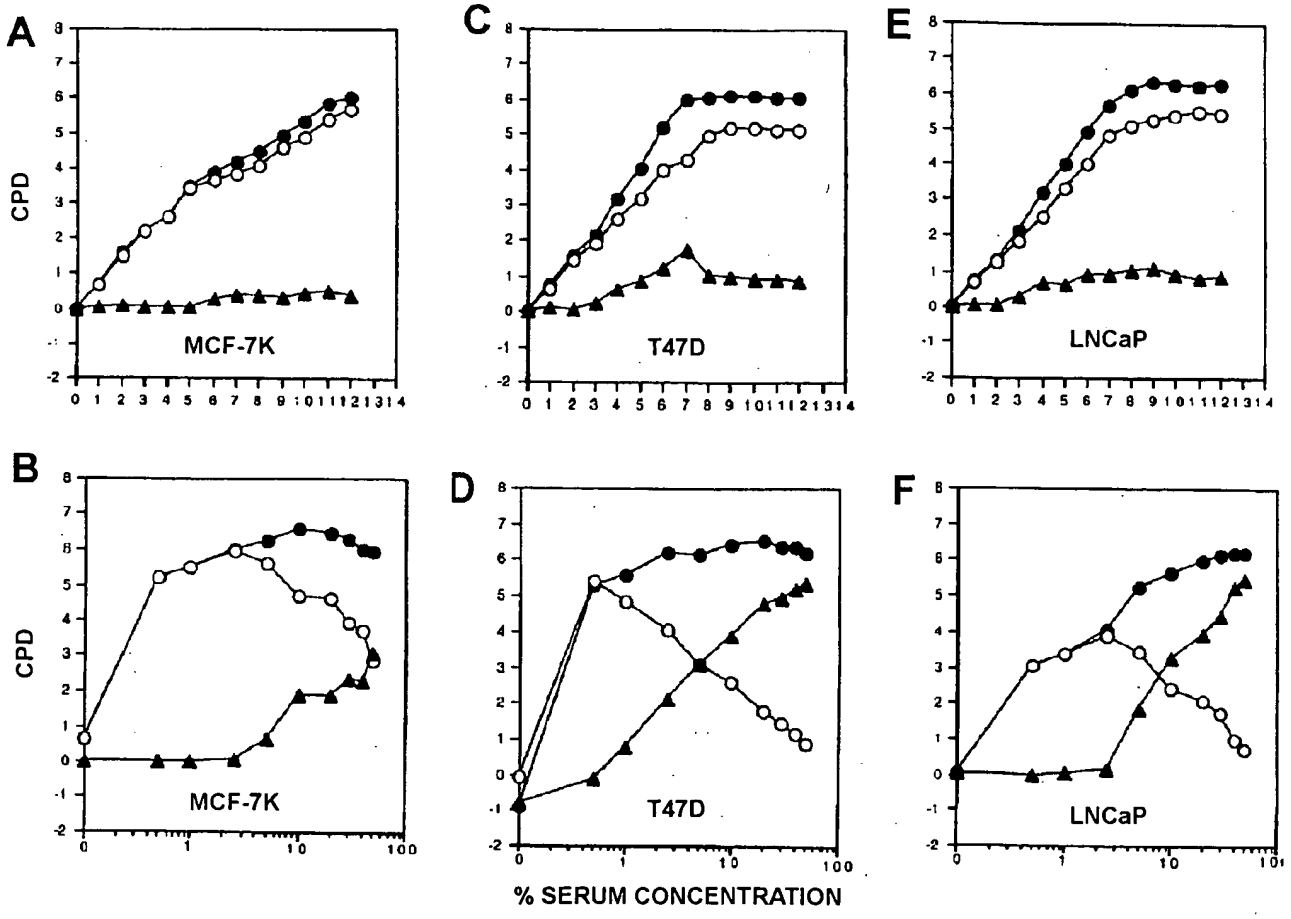
● = + 10 nM DHT

○ = STEROID FREE

▲ = ANDROGENIC EFFECT

FIGURE 56

EFFECTS OF ESTROGEN ON STEROID HORMONE-RESPONSIVE HUMAN TUMOR CELL GROWTH

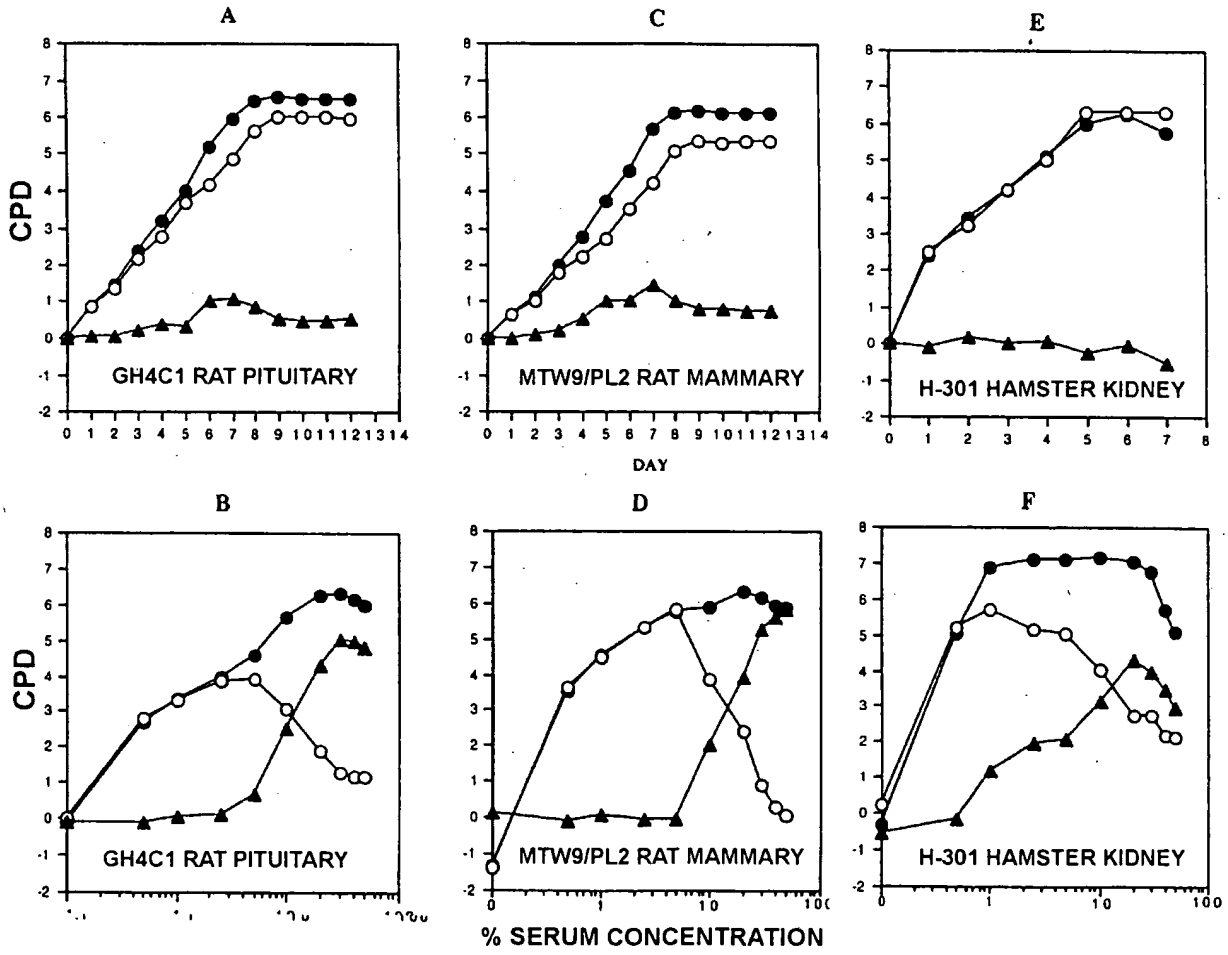


The cells were grown in serum-free defined medium and in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum.

(A) MCF-7K cell growth was measured daily in serum-free defined DDM-2MF with 10 nM E_2 (closed circles) and without steroid (open circles) E_2 . Triangles = estrogenic effect.
(B) MCF-7K cell growth measured after 12 d in D-MEM-F-12 supplemented with the designated concentrations of serum with E_2 (closed circles) and without steroid (open circles). The estrogenic effect is shown by triangles.
(C) and (D) show the same experiments as in (A) and (B), respectively, except with T47D cells.
(E) and (F) show the same experiments as in (A) and (B), respectively, except with LNCaP cells. In (E) the serum-free medium was CAPM.

FIGURE 57

EFFECTS OF ESTROGEN ON STEROID HORMONE-RESPONSIVE RODENT TUMOR CELL GROWTH

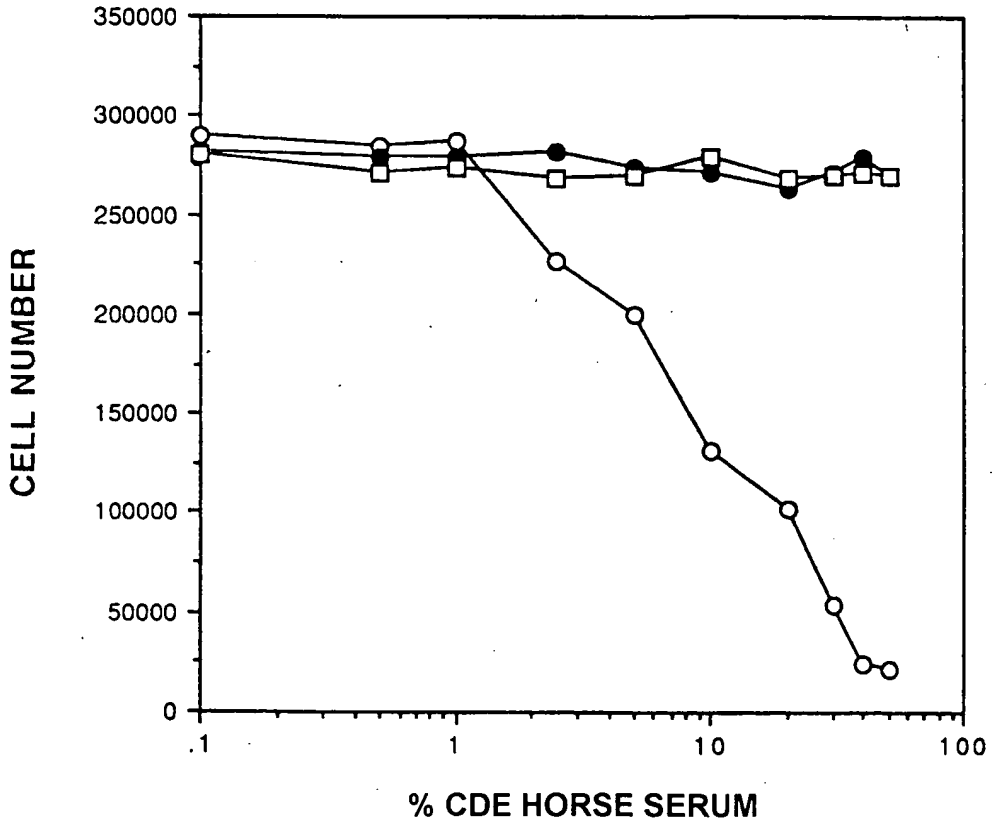


Comparison of the effects of estrogen on steroid hormone-responsive rodent tumor cell growth in serum-free defined medium and in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum.

(A) GH_4C_1 rat pituitary tumor cell growth measured daily in serum-free PCM-9 with E_2 (closed circles) and without E_2 (open circles). The estrogenic effect is shown by triangles.
(B) GH_4 C_1 cell growth measured after 9 d in D-MEM-F-12 supplemented with the designated concentrations of CDE horse serum with E_2 (closed circles) and without E_2 (open circles). The estrogenic effect is shown by triangles.
(C) and (D) show the same experiments as in (A) and (B) respectively, but with the MTW9/PL2 rat mammary tumor cells. The serum-free medium in (D) was DDM-2A.
(E) and (F) show the same experiments as in (A) and (B), respectively, except with the H-301 hamster kidney tumor cells. In (E) the serum-free medium was CAPM.

FIGURE 58

CDE HORSE SERUM TITRATION ON LNCaP GROWTH IN SERUM FREE CONDITIONS



LEGEND:

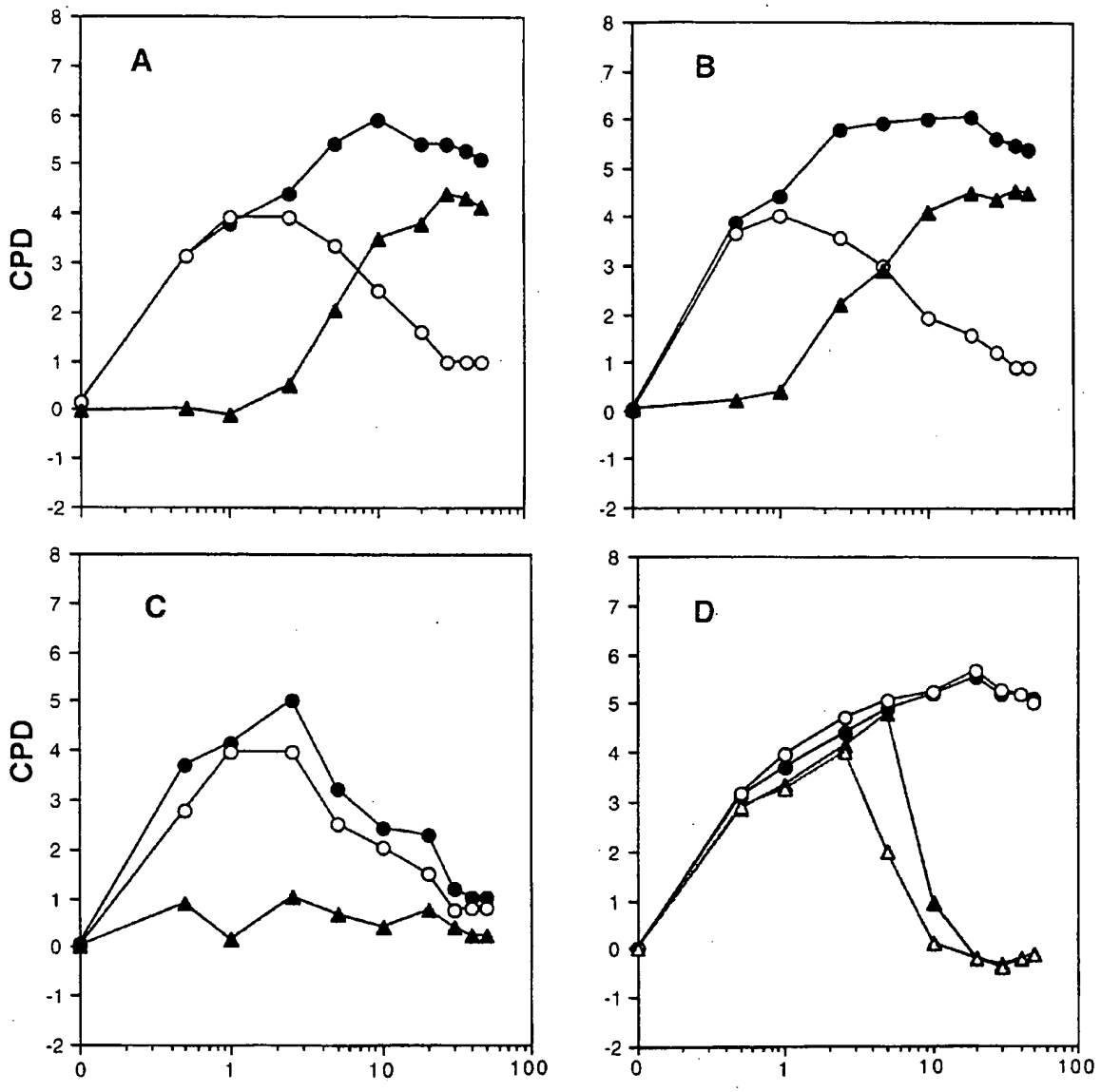
NO STEROID

+ E_2

+ DHT

FIGURE 59

THE EFFECT OF DHT, E_2, AND DES ON LNCaP CELLS GROWN IN CDE HORSE SERUM



LEGEND:

(A) Open circles = - DHT
Closed circles = + DHT
Closed trianges = Androgenic effect

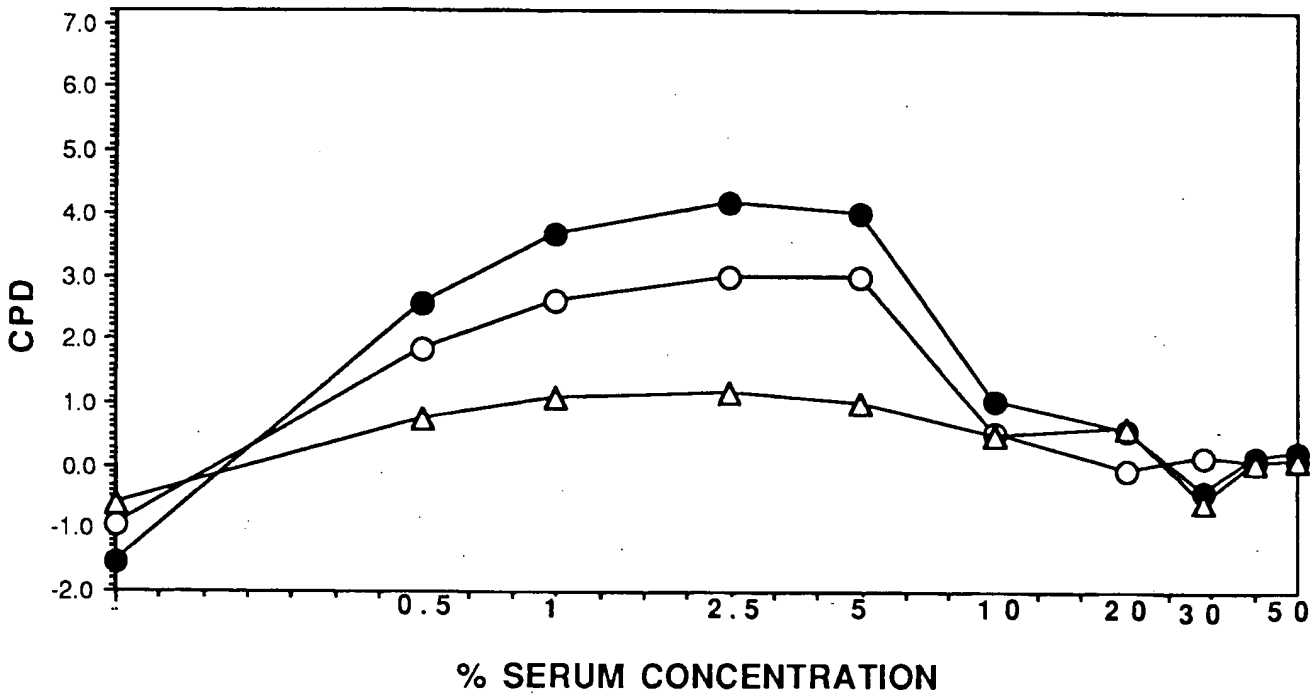
(B) Open circles = - E_2
Closed circles = + E_2
Closed triangles = Estrogenic effect

(C) Open circles = - DES
Closed circles = + DES
Closed triangles = Estrogenic effect

(D) Open circles = DHT & DES
Closed circles = E_2 & DES
Open triangles = No additions
Closed triangles = DES only

FIGURE 60

TRIS DIALYSIS OF CDE HORSE SERUM AND ASSAY WITH MTW9/PL2 CELLS



LEGEND:

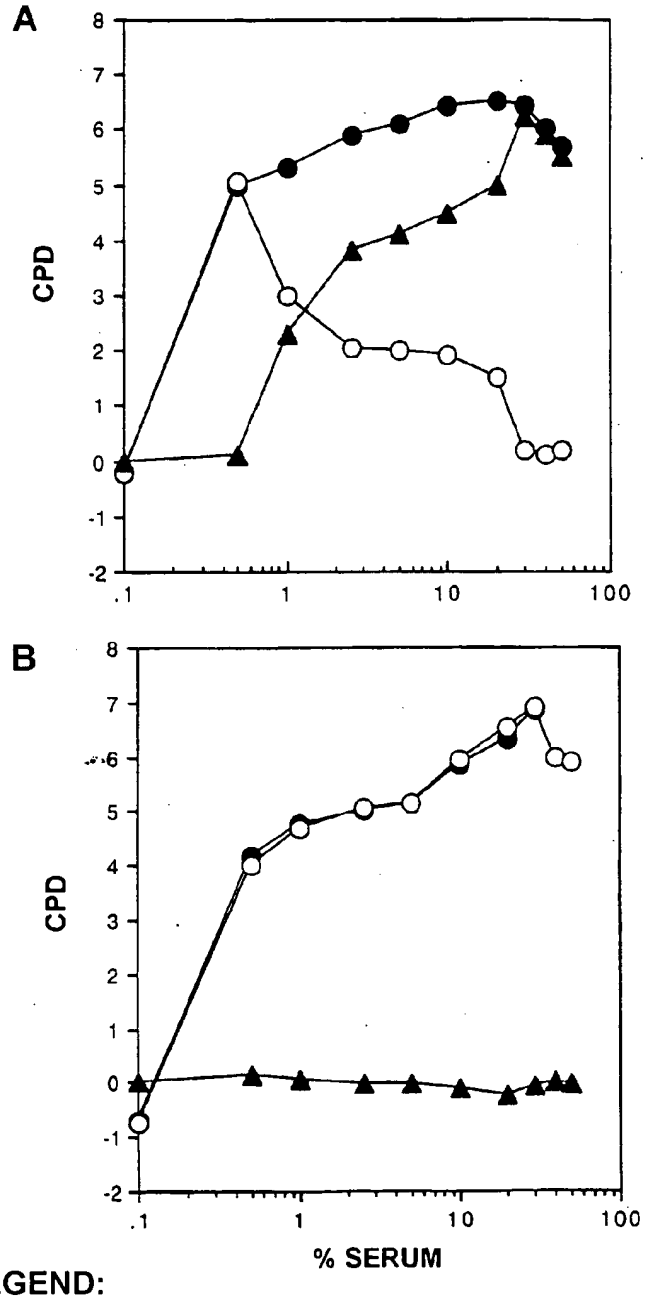
= + E_2

= - E_2

= Estrogenic effect

FIGURE 61

ULTRAFILTRATION OF CDE HORSE SERUM AND ESTROGENIC EFFECTS WITH MTW9/PL2 CELLS



LEGEND:

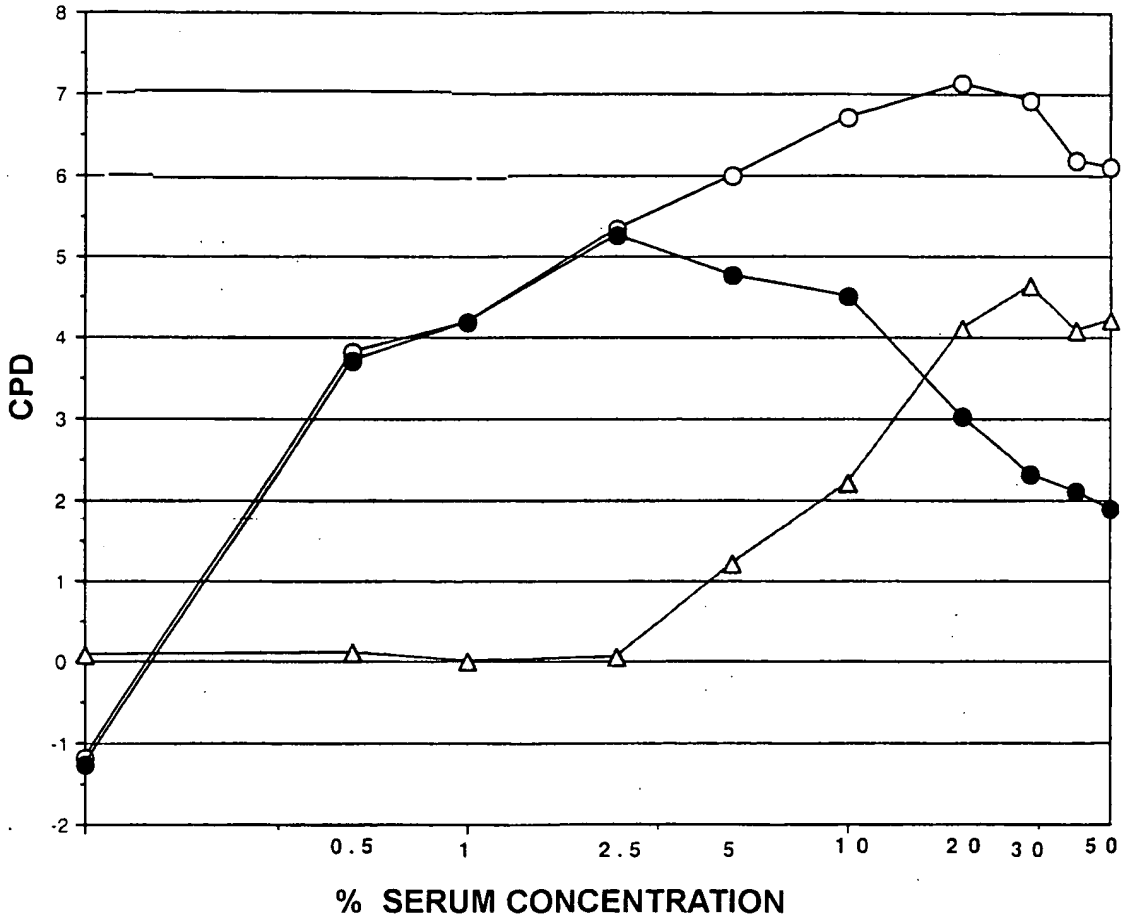
(A) RETENTATE FROM AMICON MEMBRANE

(B) FILTRATE FROM AMICON MEMBRANE

Open circles = $-E_2$
Closed circles = $+E_2$
Closed triangles = Estrogenic effect

FIGURE 62

CDE HORSE SERUM TREATED AT 50° C FOR 30 MINUTES AND ASSAYED WITH MTW9/PL2 CELLS



LEGEND:

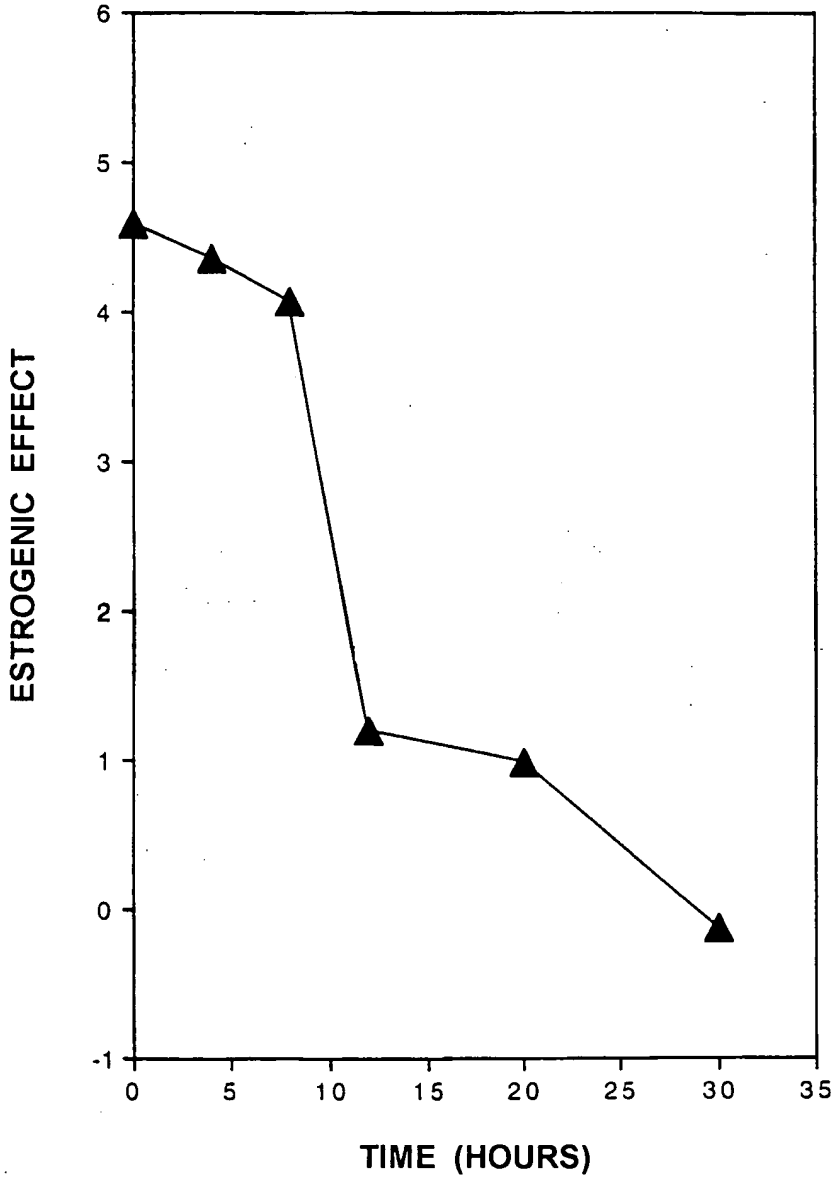
—○— = + E_2

—●— = − E_2

—△— = Estrogenic effect

FIGURE 63

EFFECT OF 50° C INCUBATION ON ESTROGENIC EFFECT WITH MTW9/PL2

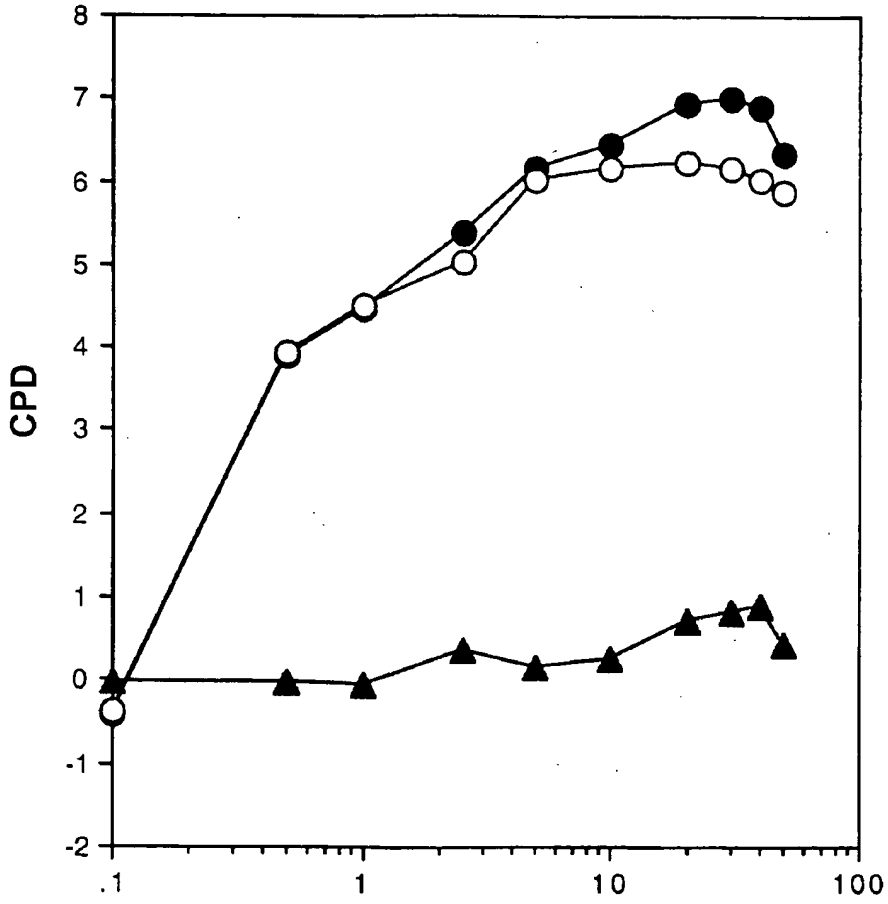


LEGEND:

Closed triangles = Estrogenic effect

FIGURE 64

CDE HORSE SERUM INCUBATION AT 50° C
FOR 20 HOURS AND ASSAYED WITH MTW9/PL2



LEGEND:

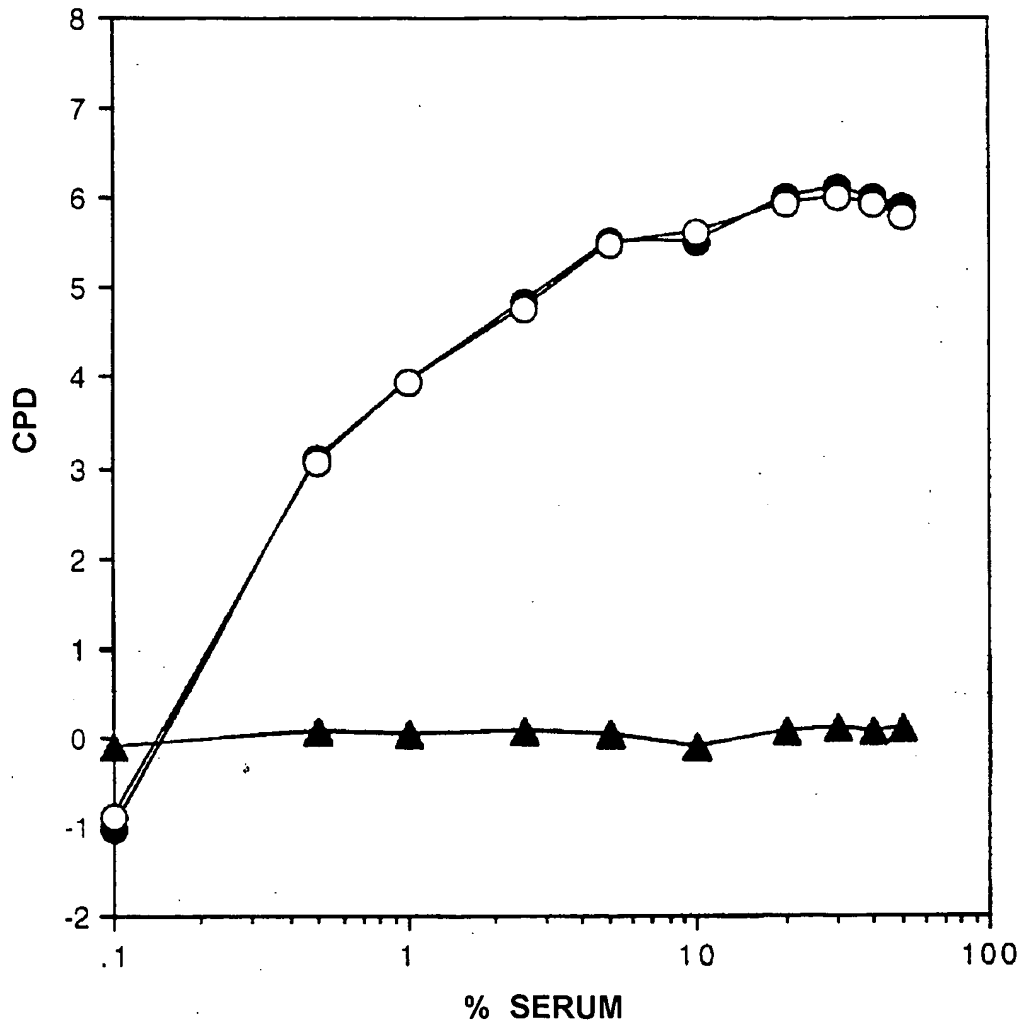
Open circles = $- E_2$

Closed circles = $+ E_2$

Closed triangles = Estrogenic effect

FIGURE 65

CDE HORSE SERUM INCUBATED AT 60° C FOR 90 MINUTES AND ASSAYED WITH MTW9/PL2 CELLS



LEGEND:

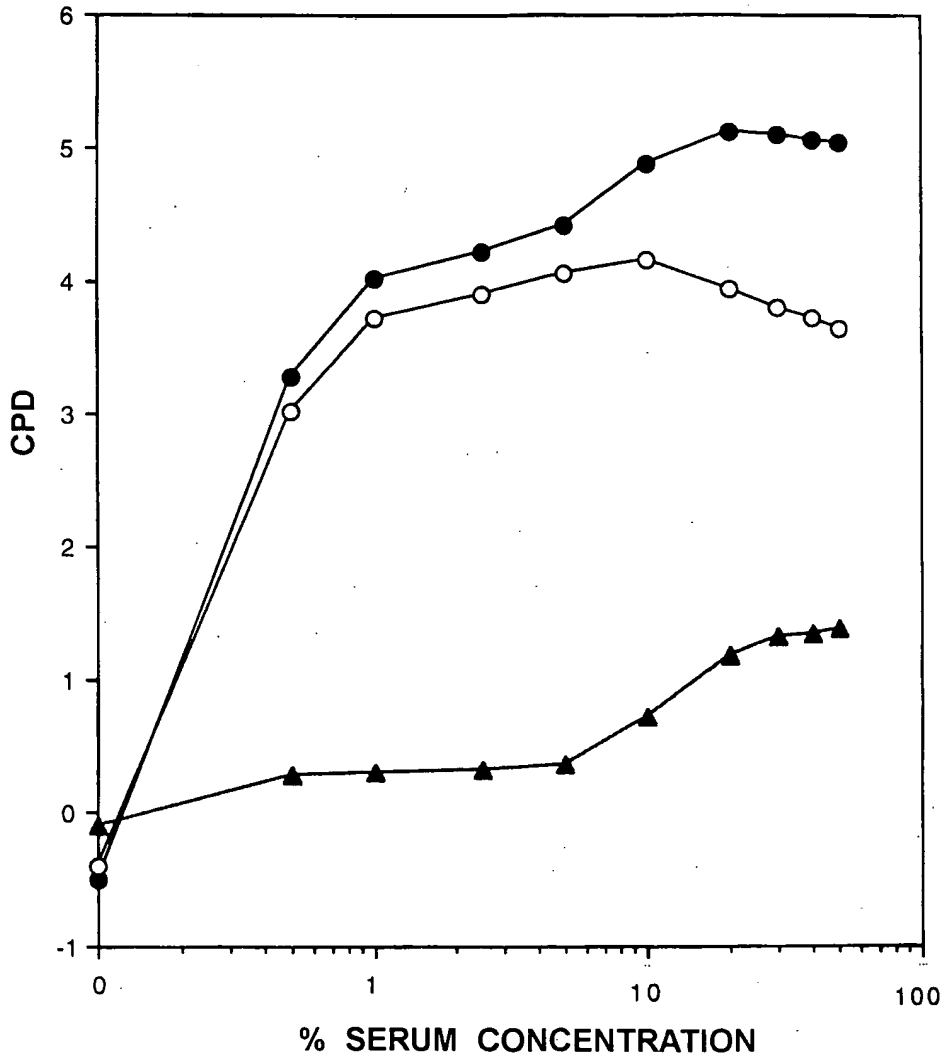
Open circles = − E_2

Closed circles = + E_2

Closed triangles = Estrogenic effect

FIGURE 66

AFFI-GEL BLUE TREATMENT OF CDE HORSE SERUM AND ASSAY WITH MTW9/PL2 CELLS



LEGEND:

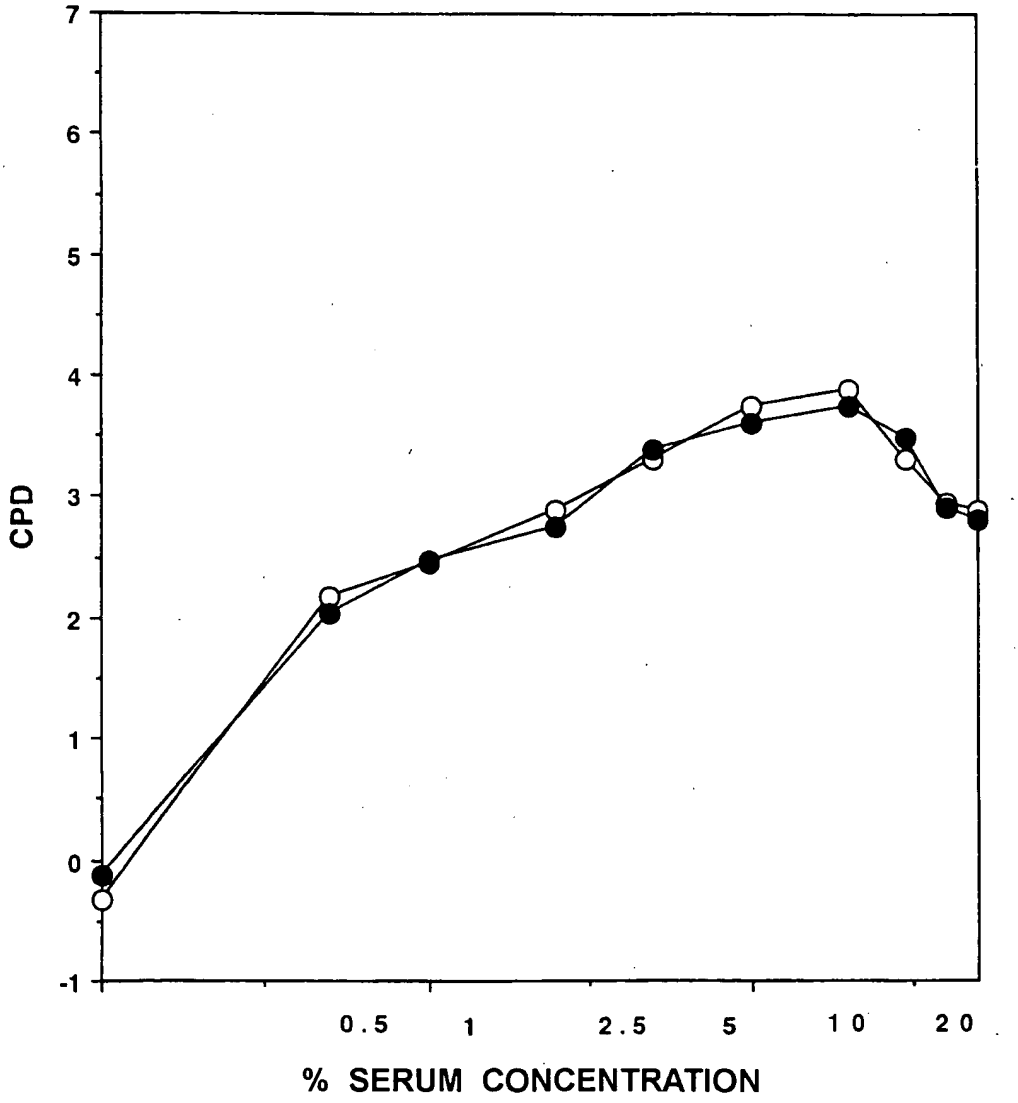
Open circles = – E_2

Closed circles = + E_2

Closed triangles = Estrogenic effect

FIGURE 67

DIALYSIS OF CDE HORSE SERUM AGAINST 6M UREA AND ASSAY WITH MTW9/PL2 CELLS



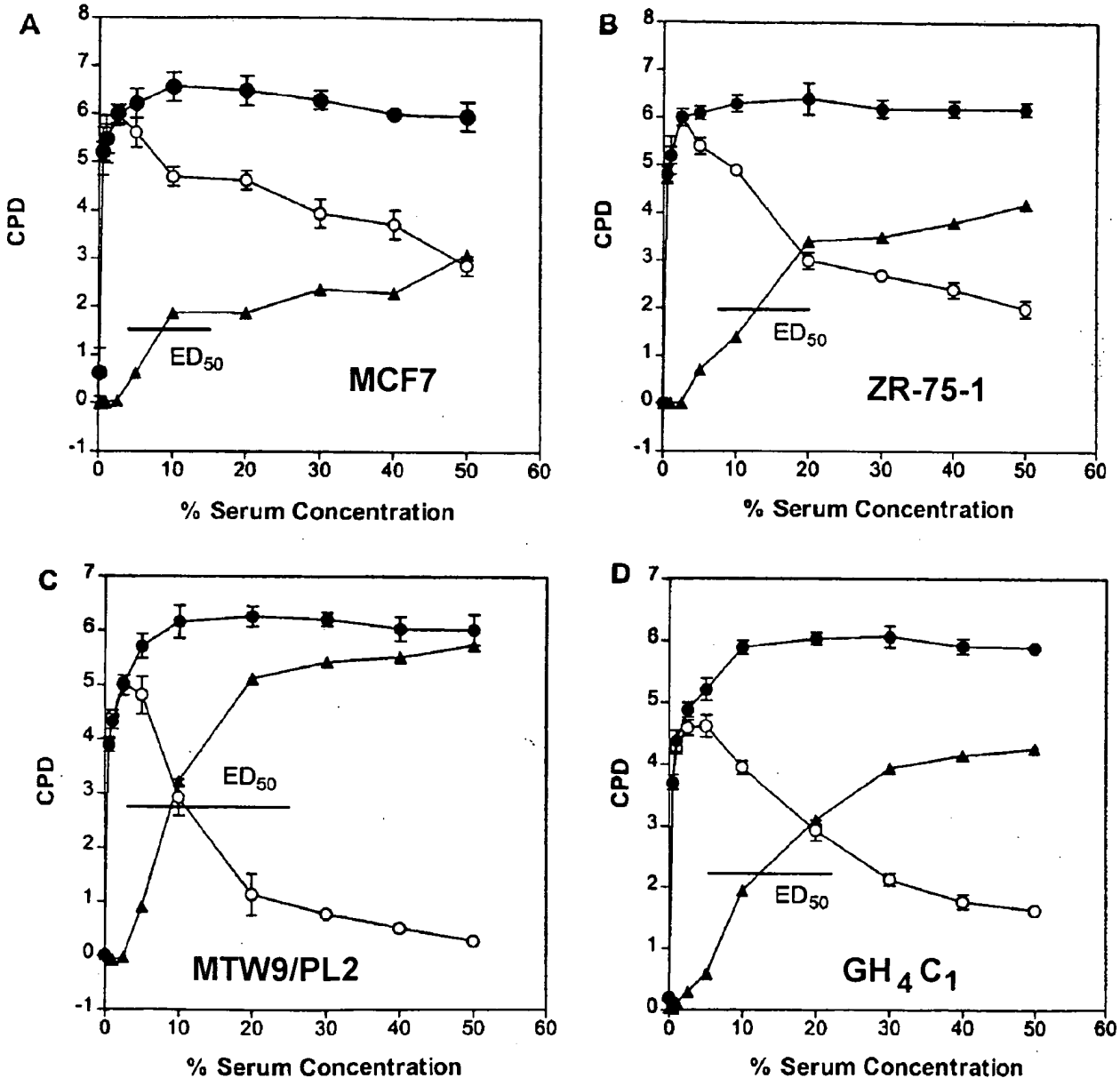
LEGEND:

—○— = + E_2

—●— = − E_2

FIGURE 68

ED_{50} MEASUREMENTS OF THE ESTROGENIC EFFECTS OF CDE HORSE SERUM WITH VARIOUS CELL LINES



LEGEND:

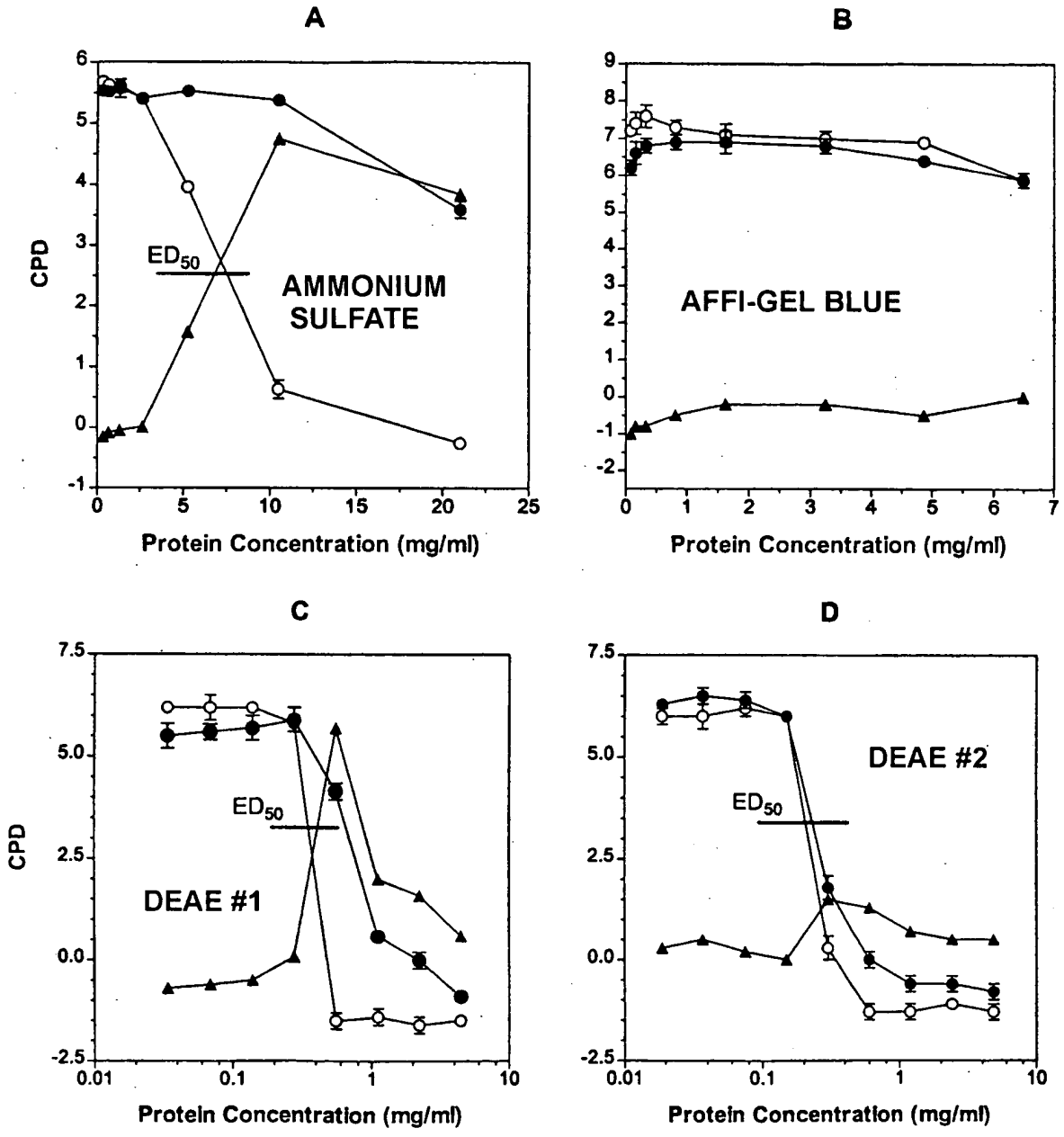
Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 69

ASSAY OF ESTROGENIC ACTIVITY (ED_{50}) OF CHROMATOGRAPHIC POOLS



LEGEND:

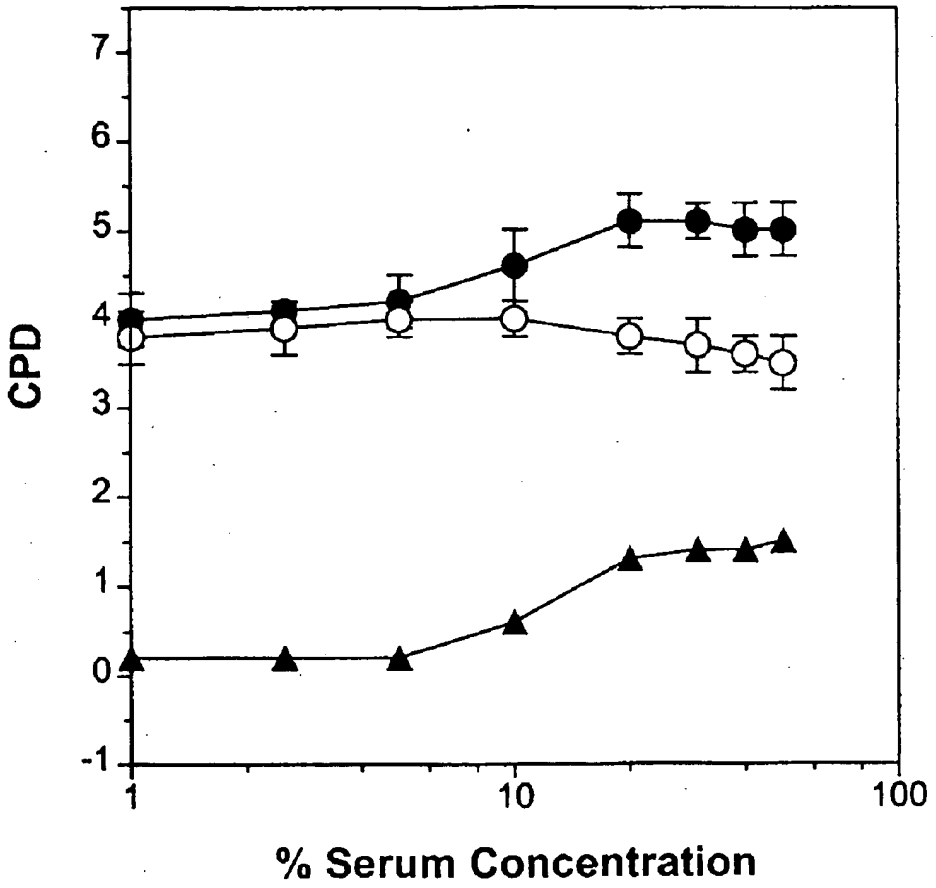
Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 70

AFFI-GEL BLUE BYPASS FRACTION ASSAYED WITH MTW9/PL2 CELLS



LEGEND:

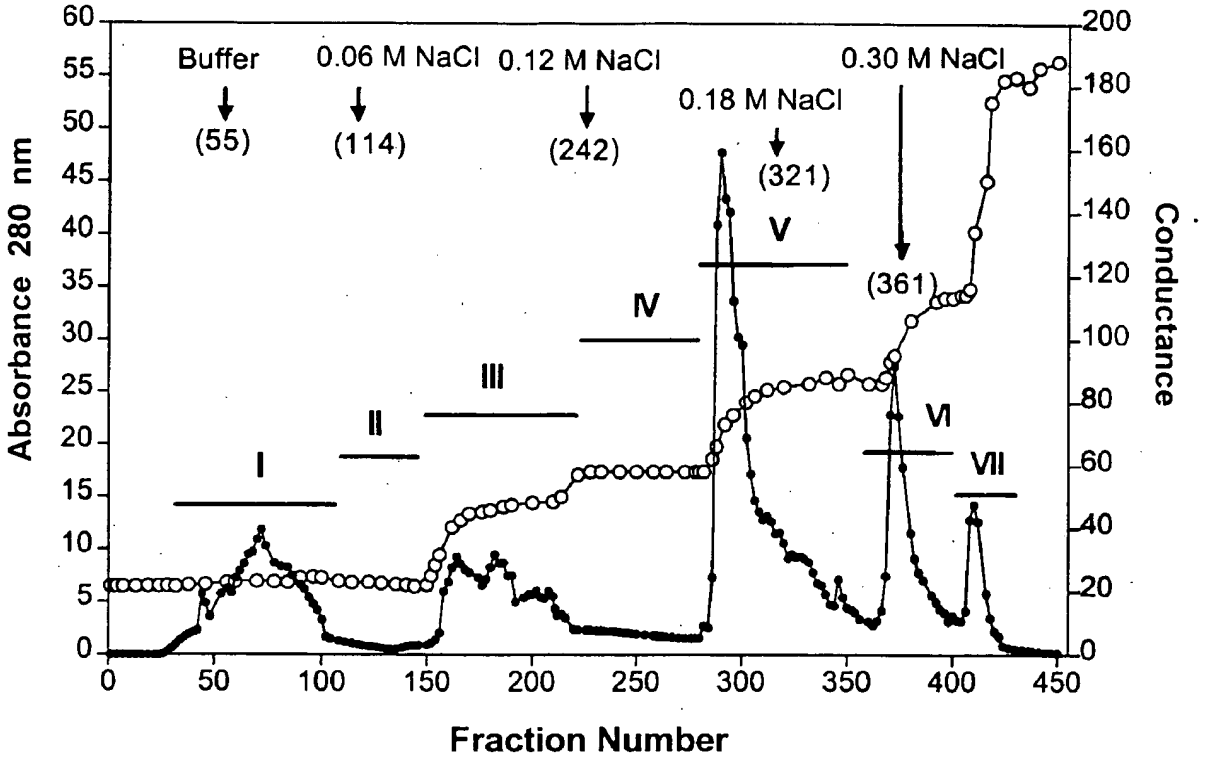
Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 71

DEAE SEPHAROSE CHROMATOGRAPHY OF CDE HORSE SERUM



LEGEND:

BARS = FRACTION POOLS

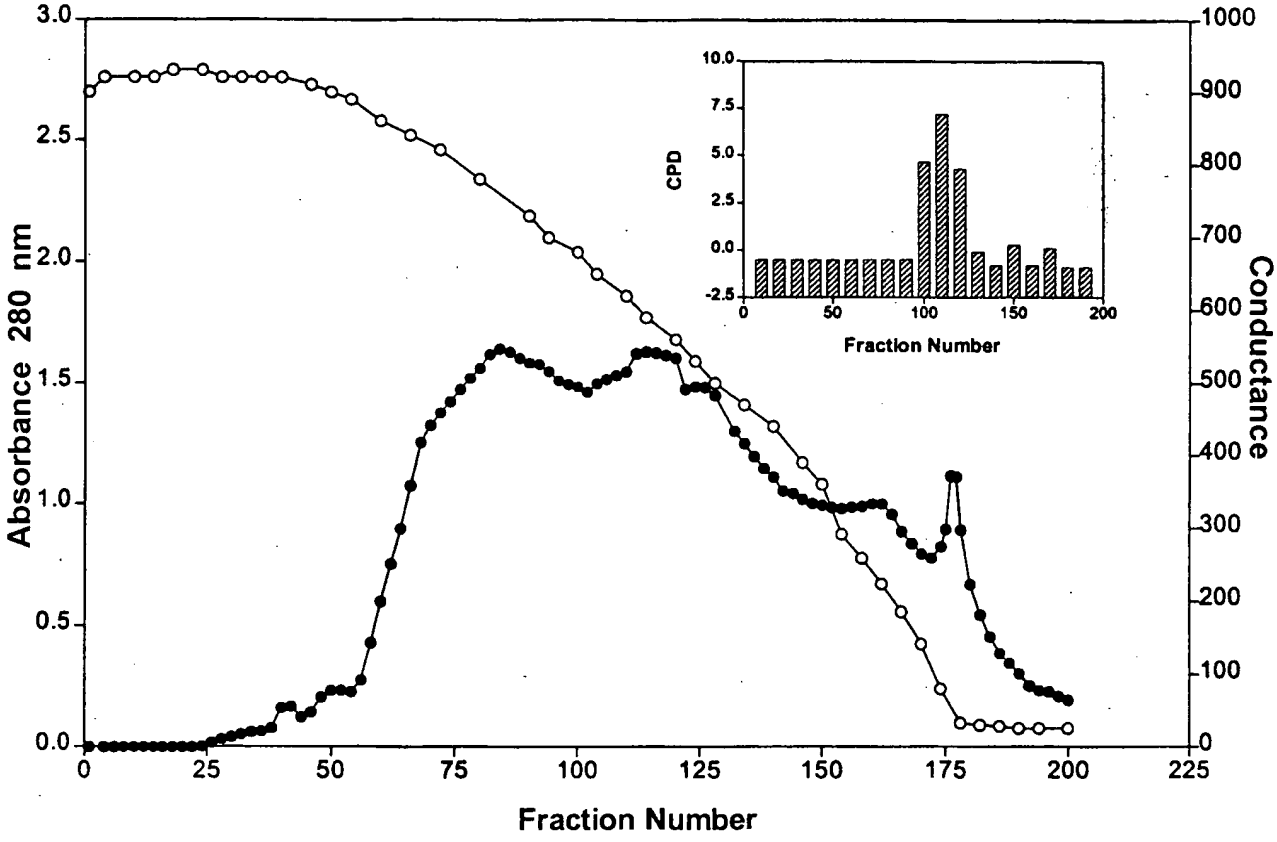
ARROWS = BUFFER CHANGES

Closed circles = Absorbance at 280 nm

Open circles = Conductance

FIGURE 72

THE ELUTION PROFILE OF PHENYL SEPHAROSE WITH THE DEAE IV POOL



INSERT: Estrogenic activity with MTW9/PL2

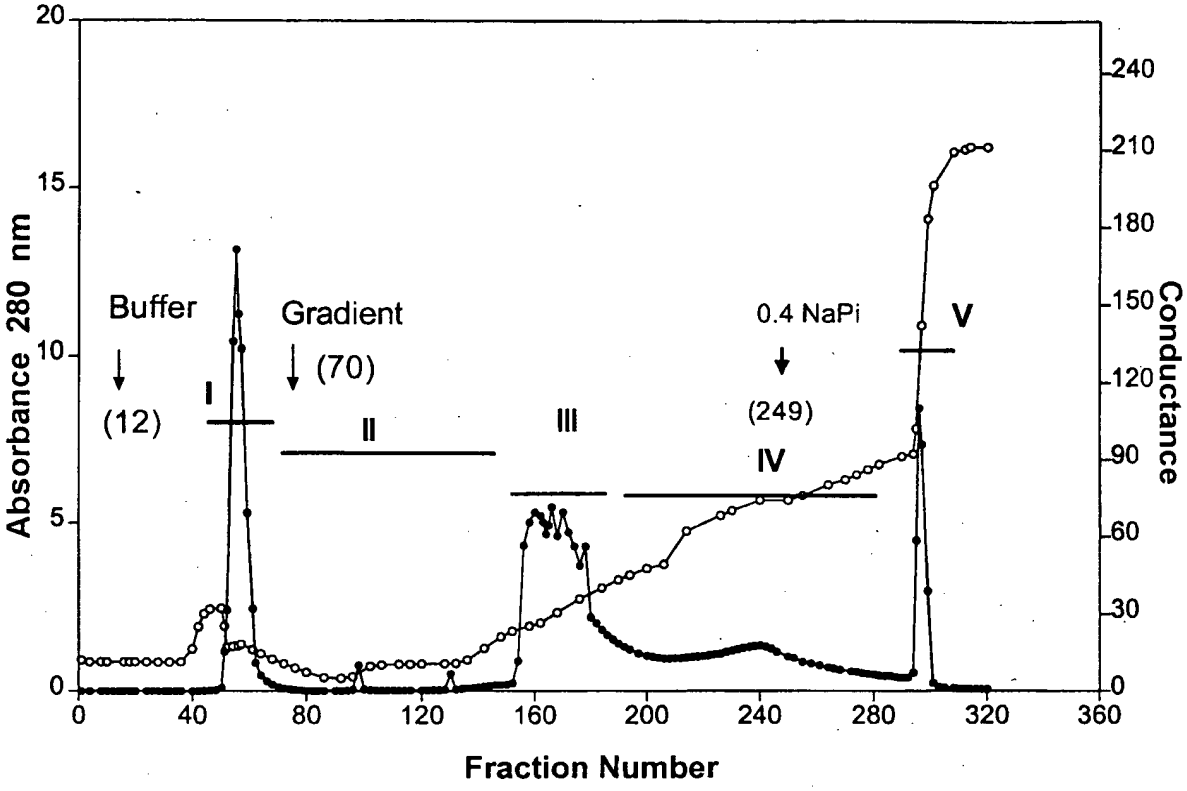
LEGEND:

Closed circles = Absorbance 280 nm

Open circles = Conductance

FIGURE 73

HTP BIO-GEL CHROMATOGRAPHY OF DEAE POOL IV



BARS = FRACTION POOLS

ARROWS = BUFFER CHANGES

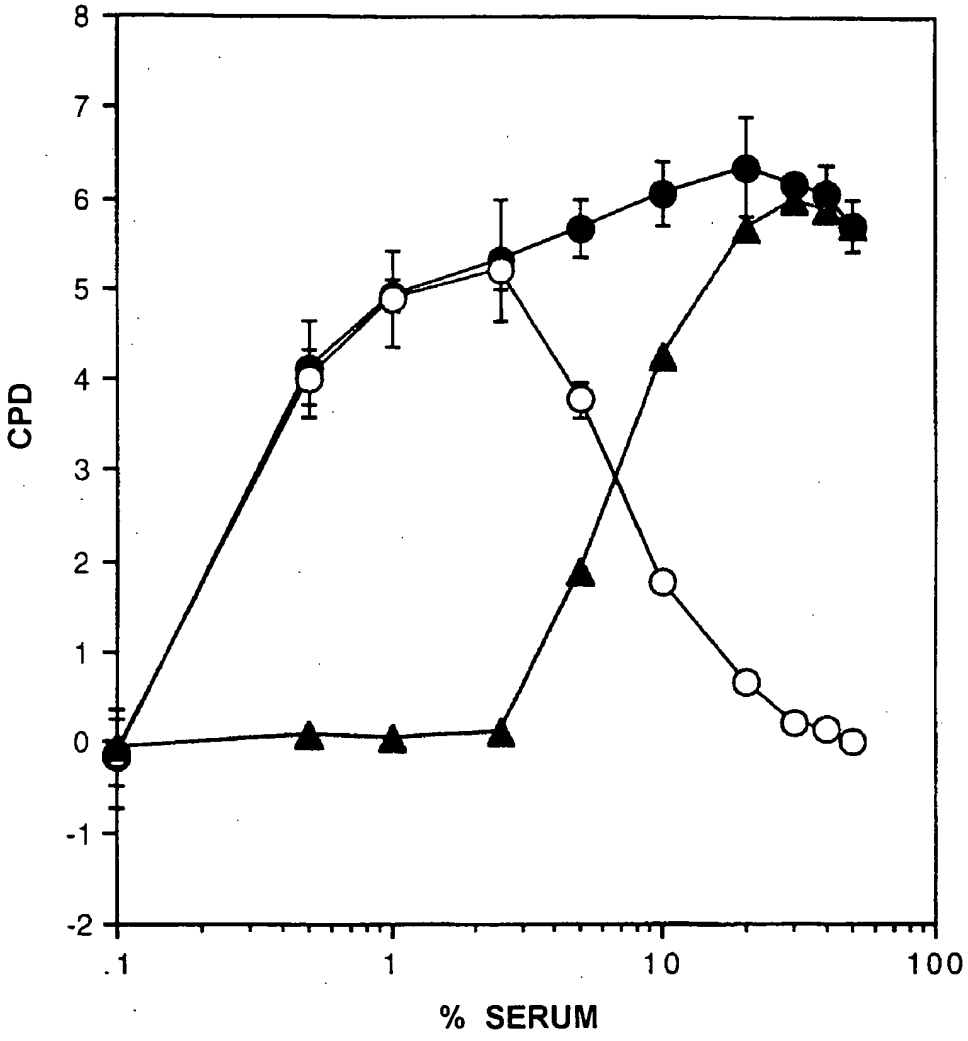
LEGEND:

Open circles = Conductance

Closed circles = Absorbance

FIGURE 74

DIALYSIS OF CDE HORSE SERUM AGAINST TRIS BUFFER CONTAINING CALCIUM



LEGEND:

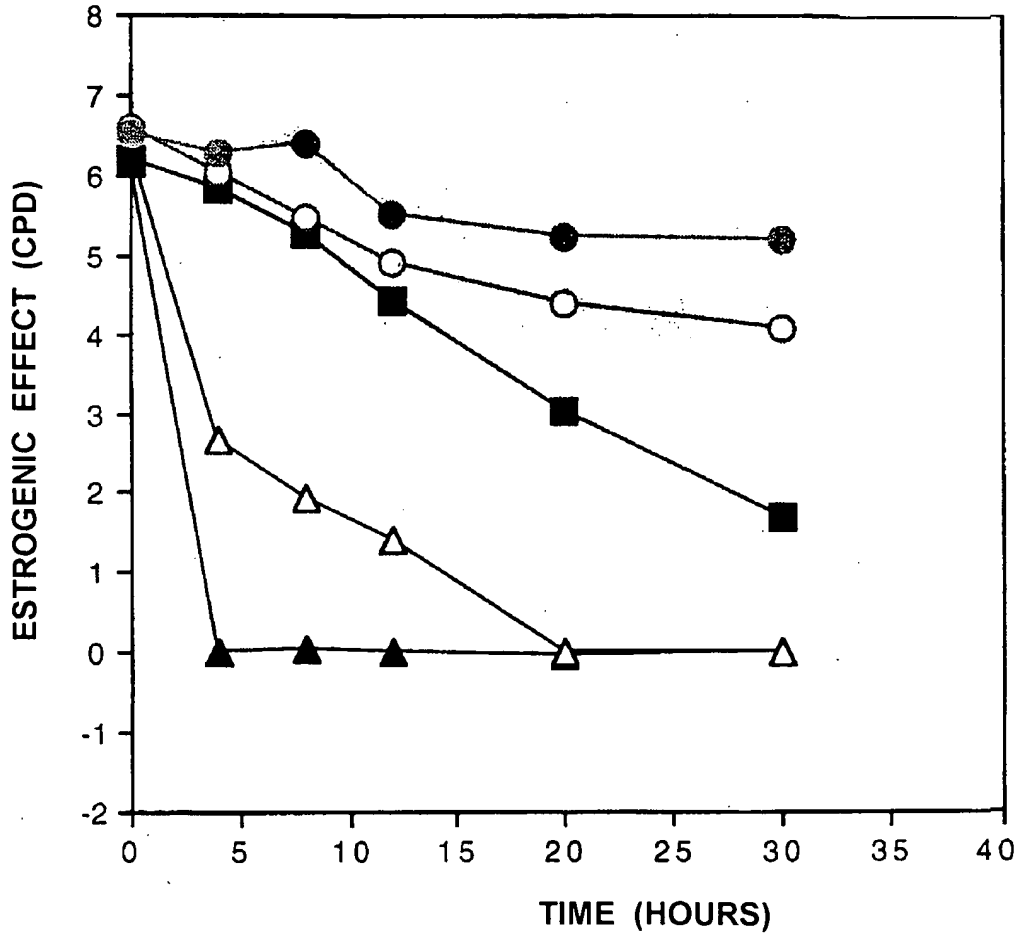
Open circles = − E_2

Closed circles = + E_2

Closed triangles = Estrogenic effect

FIGURE 75

THE EFFECT OF CALCIUM ON THE HEAT STABILITY OF THE INHIBITOR IN CDE HORSE SERUM (MTW9/PL2 CELLS)



LEGEND:

= Chelex treatment only

= CDE horse serum

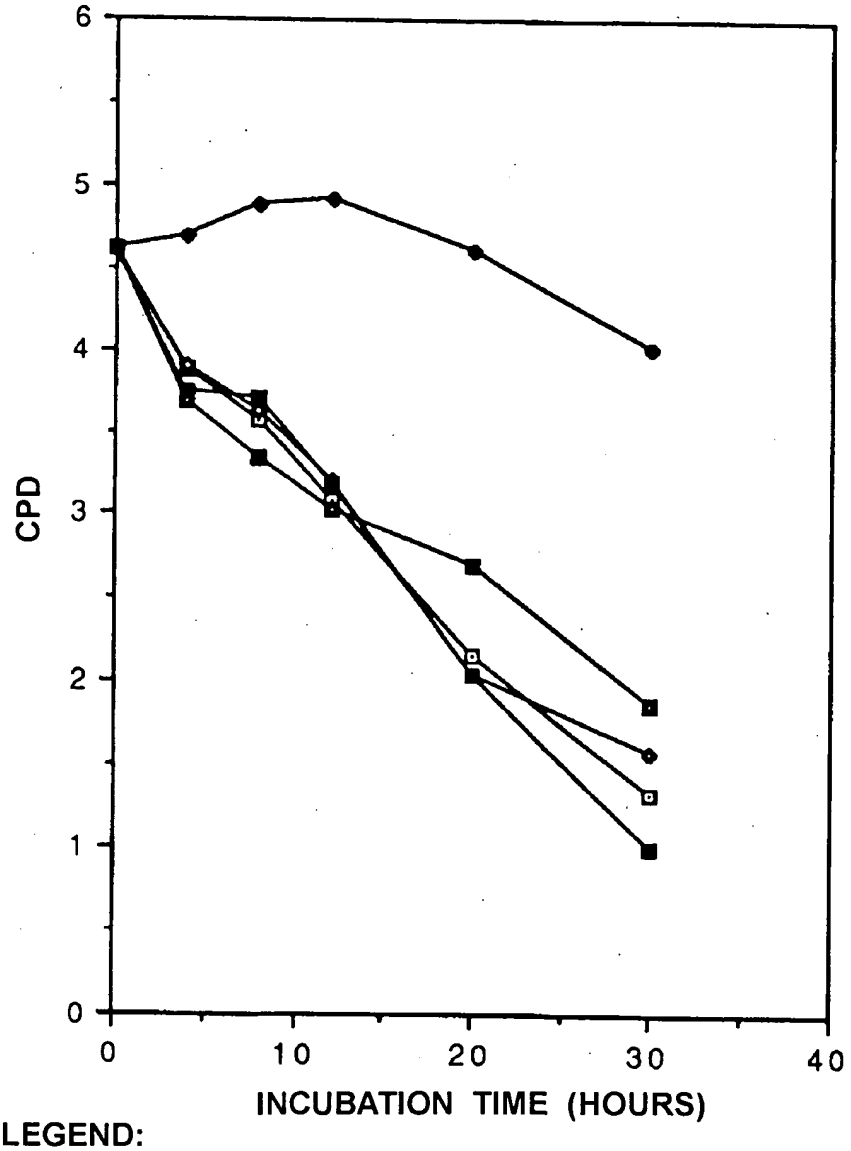
= Chelex and 1 mM calcium chloride

= Chelex and 10 mM calcium chloride

= Chelex and 50 mM calcium chloride

FIGURE 76

PROTECTIVE EFFECT OF METAL IONS ON CHELEX TREATED CDE HORSE SERUM INCUBATED AT 37° C AND ASSAYED WITH MTW9/PL2 CELLS

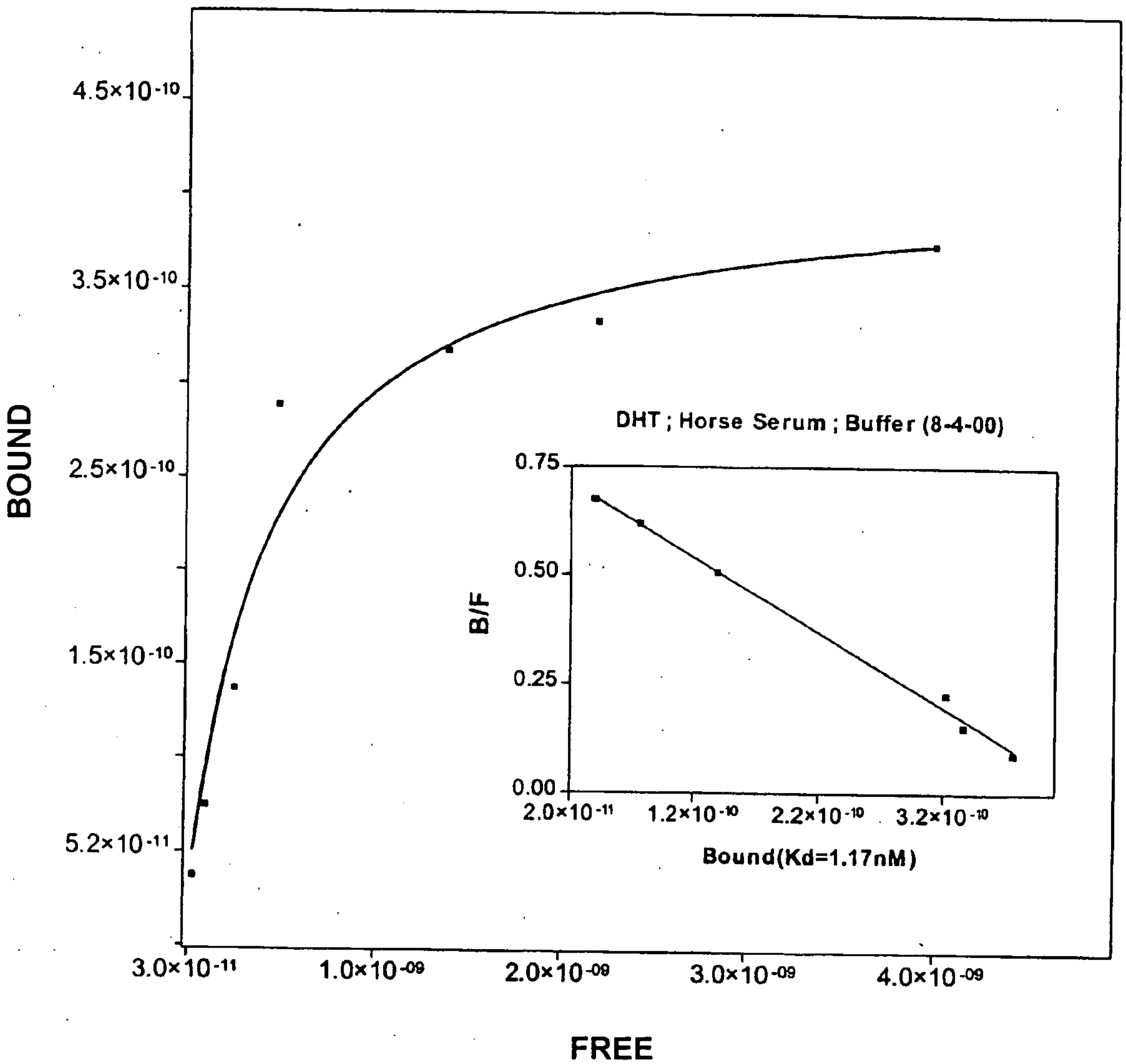


LEGEND:

- Chelex treated serum
- Chelex treated serum + 10 mM Calcium
- Chelex treated serum + 50 uM Manganese
- Chelex treated serum + 100 uM Magnesium
- Chelex treated serum + 10 uM Zinc

FIGURE 77

LABELED DHT BINDING TO CDE HORSE SERUM SATURATION ANALYSIS AND SCATCHARD PLOT



INSERT:

Scatchard analysis of DHT binding

FIGURE 78

EFFECT OF CALCIUM ON ESTROGENIC EFFECT (A) AND LABELED STEROID HORMONE BINDING (B)

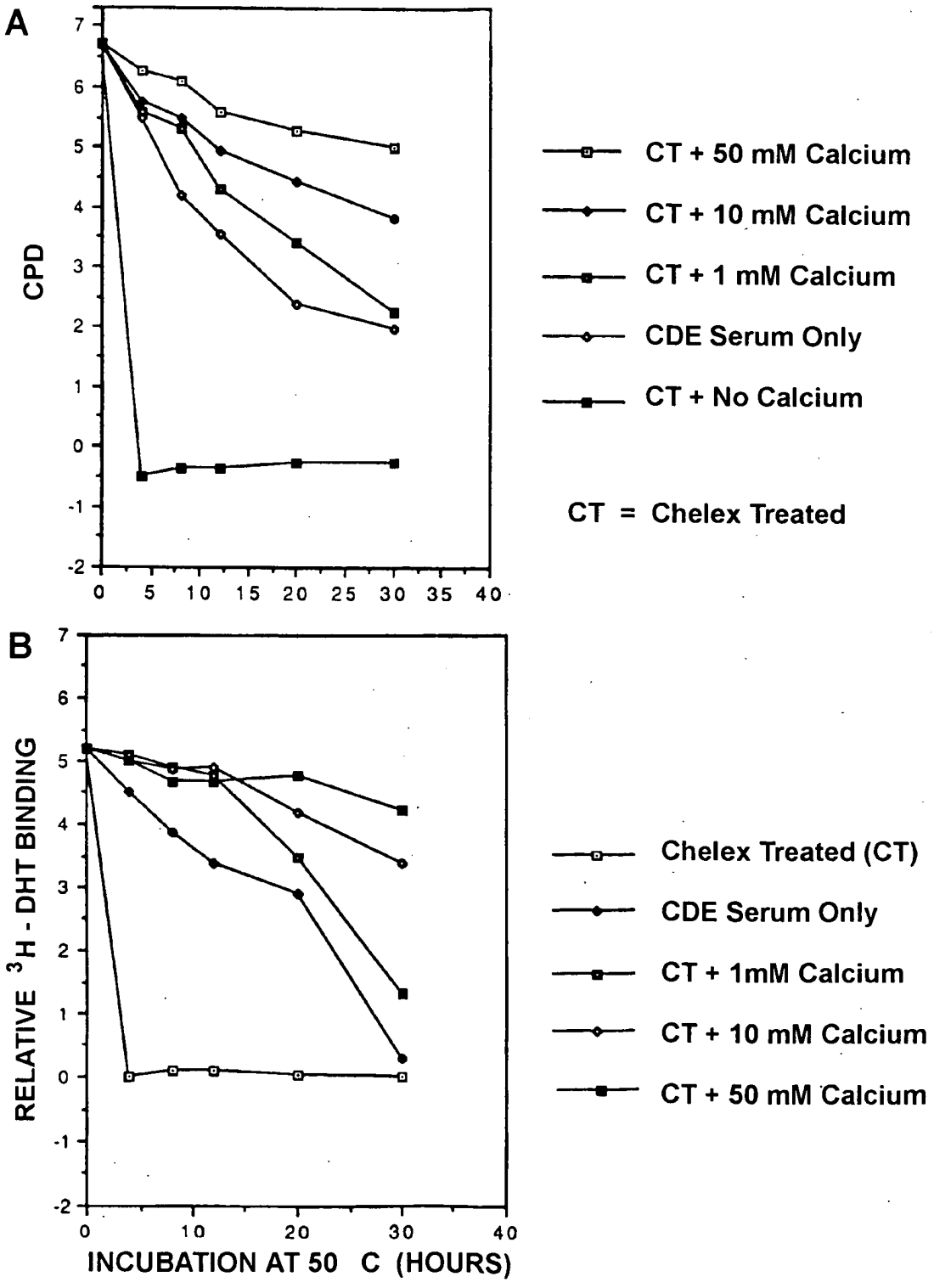


FIGURE 79

ANTI - HUMAN SHBG PRECIPITATION OF THE LABELED DHT BINDING ACTIVITY (A) AND THE ESTROGENIC EFFECT IN CDE HORSE SERUM (B)

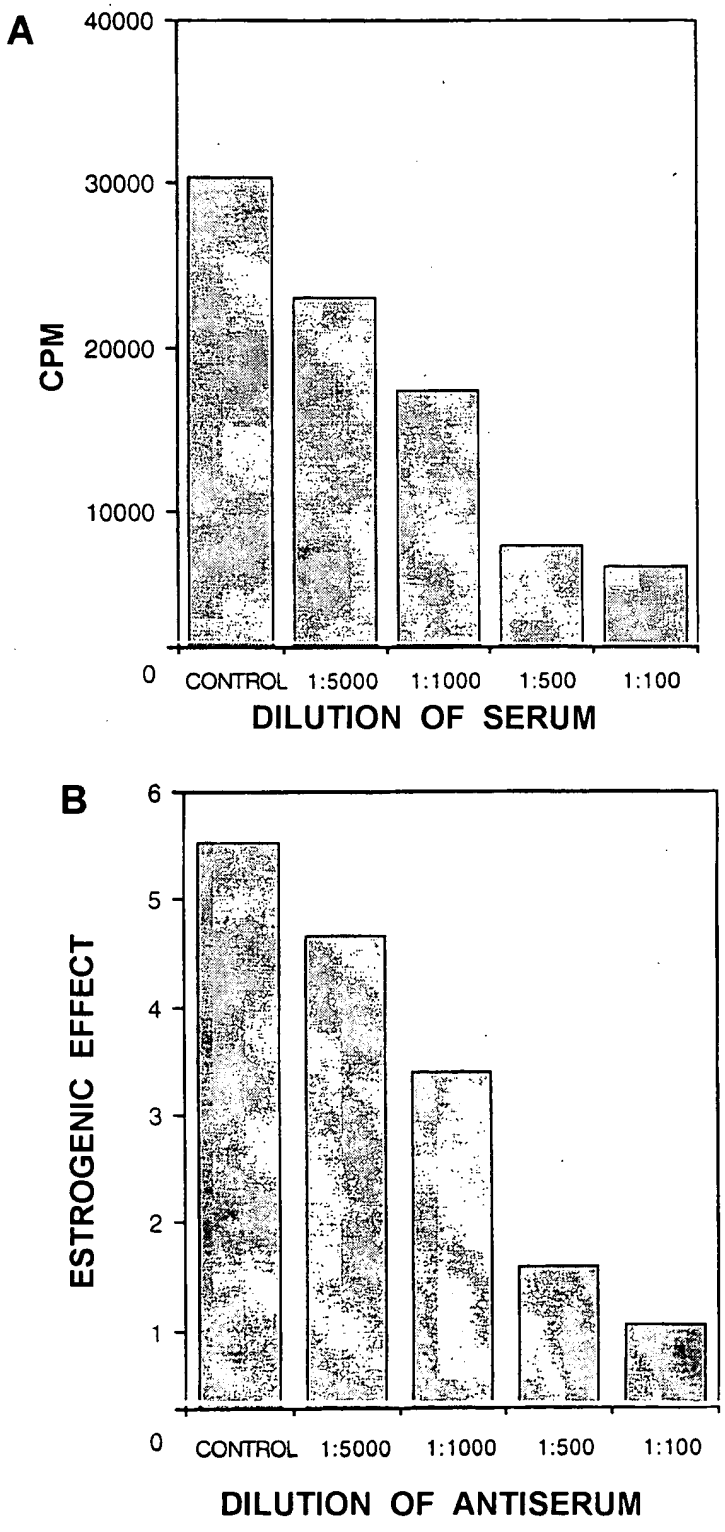
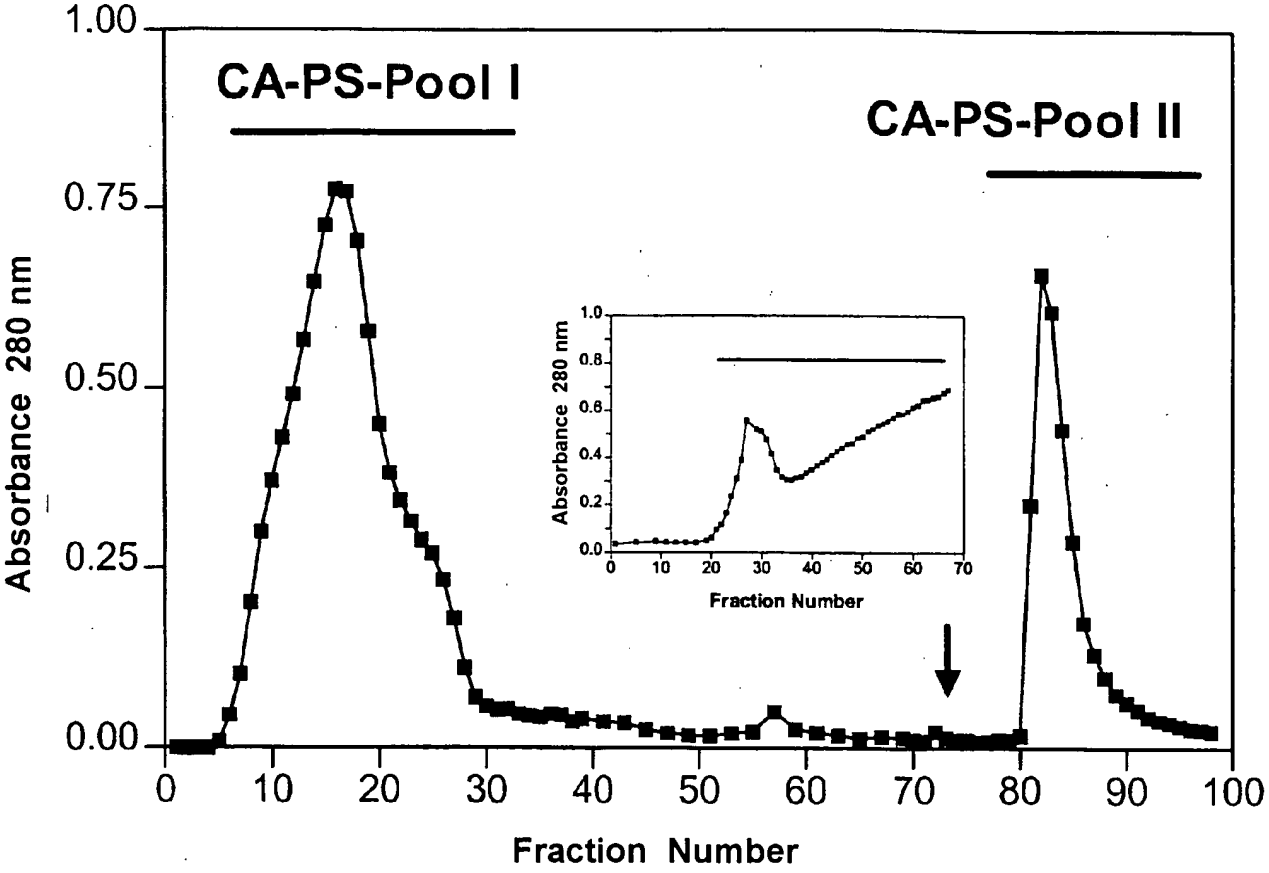


FIGURE 80

PHENYL SEPHAROSE ELUTION OF

CBG (CA-PS-POOL 1) AND SHBG-LIKE (CA-PS-POOL 11)



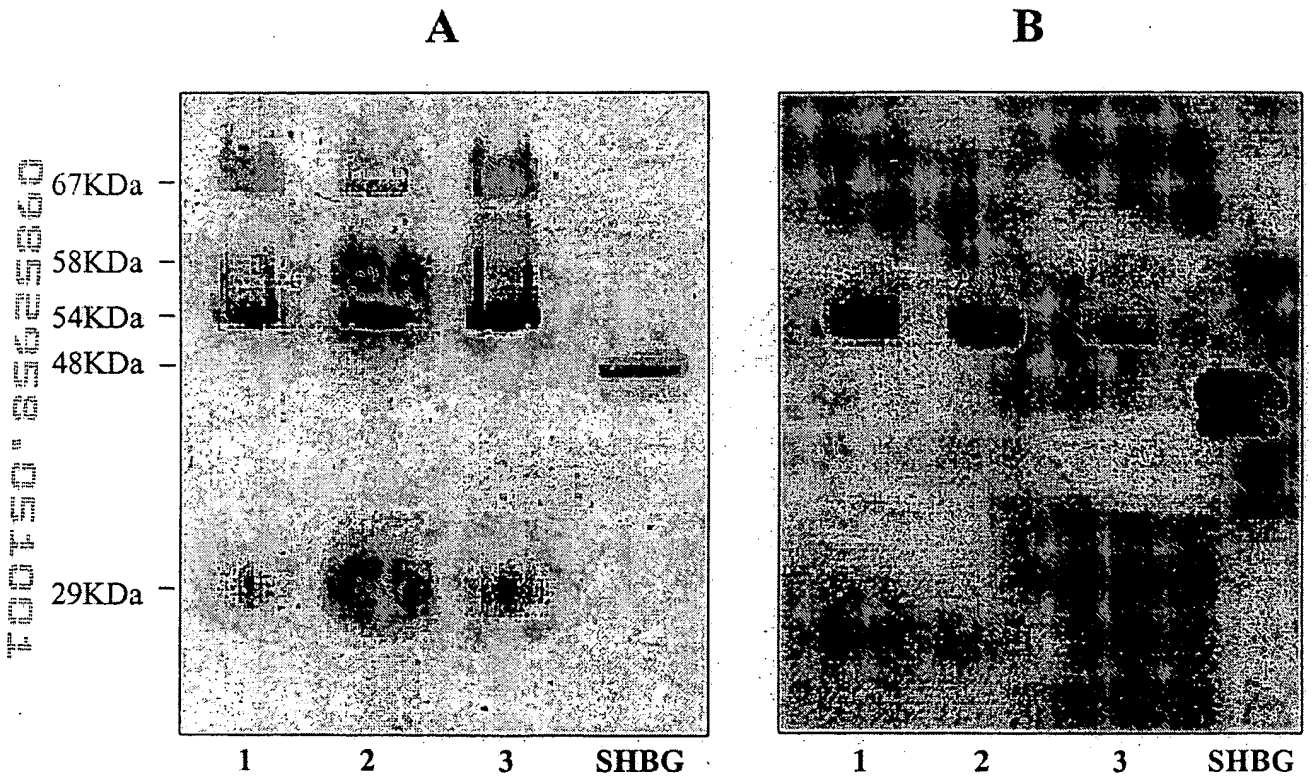
ARROW = ELUTION WITH 40% ETHYLENE GLYCOL

INSERT: CORTISOL AFFINITY COLUMN ELUTION

BARS = POOLED ACTIVE FRACTION

FIGURE 81

SDS PAGE (A) AND WESTERN ANALYSIS (B) OF THREE PREPARATIONS OF CA-PS-POOL II VS HUMAN SHBG

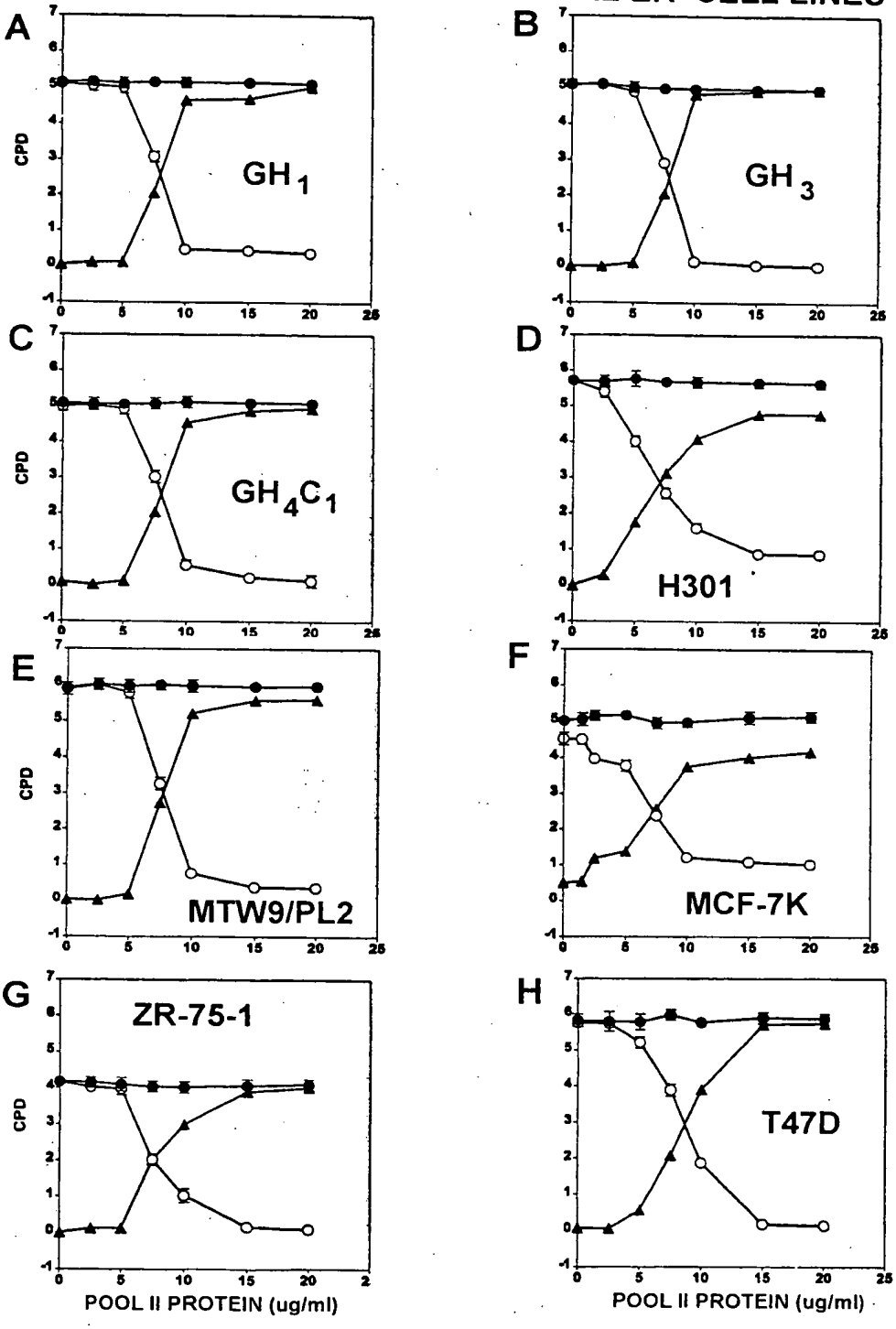


LANES 1, 2, AND 3 = 10 ug each of CA-PS-POOL II

LANE "SHBG" = 10 mg of purified protein

FIGURE 82

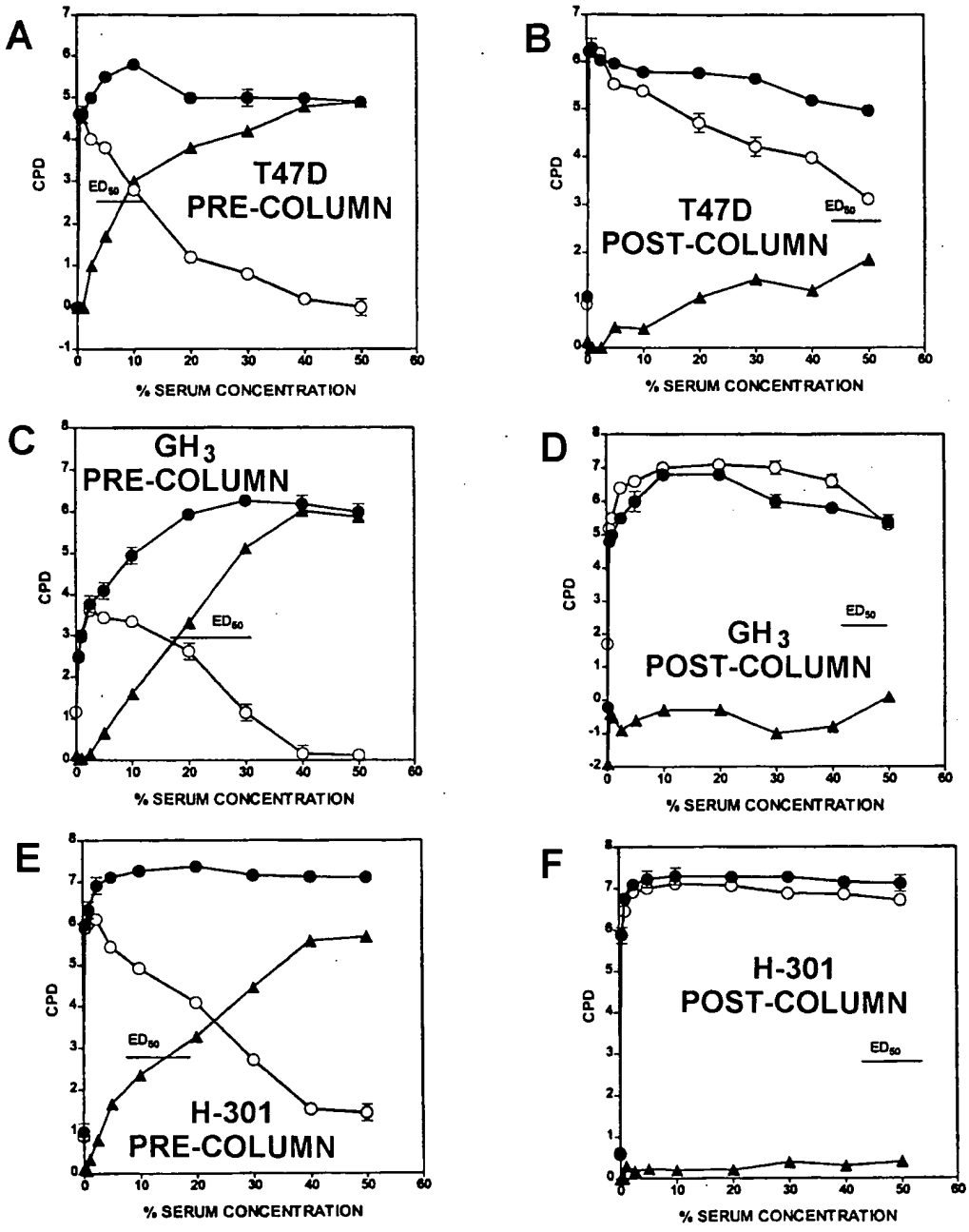
ASSAY OF CA-PS-POOL II ESTROGEN REVERSIBLE INHIBITORY ACTIVITY WITH SEVERAL ER CELL LINES



LEGEND: Open circles = $-E_2$
Closed circles = $+E_2$
Closed triangles = Estrogenic effect

FIGURE 83

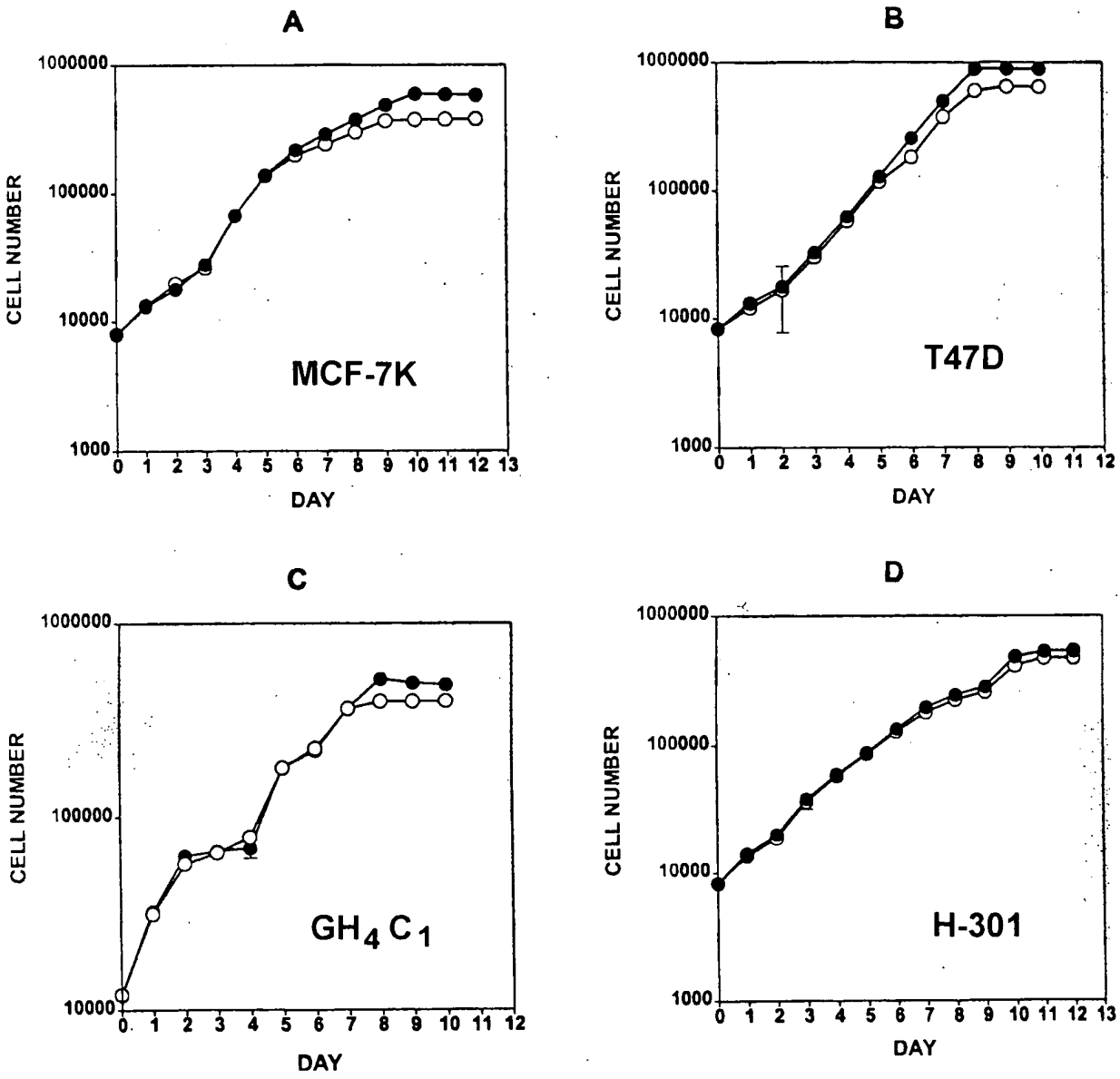
CORTISOL-AGAROSE AFFINITY REMOVAL OF THE INHIBITOR FROM CDE-SERUM



LEGEND: Open circles = $- E_2$
Closed circles = $+ E_2$
Closed triangles = Estrogenic effect

FIGURE 84

GROWTH OF ER$^+$ CELL LINES IN SERUM-FREE MEDIUM $\pm$ E_2



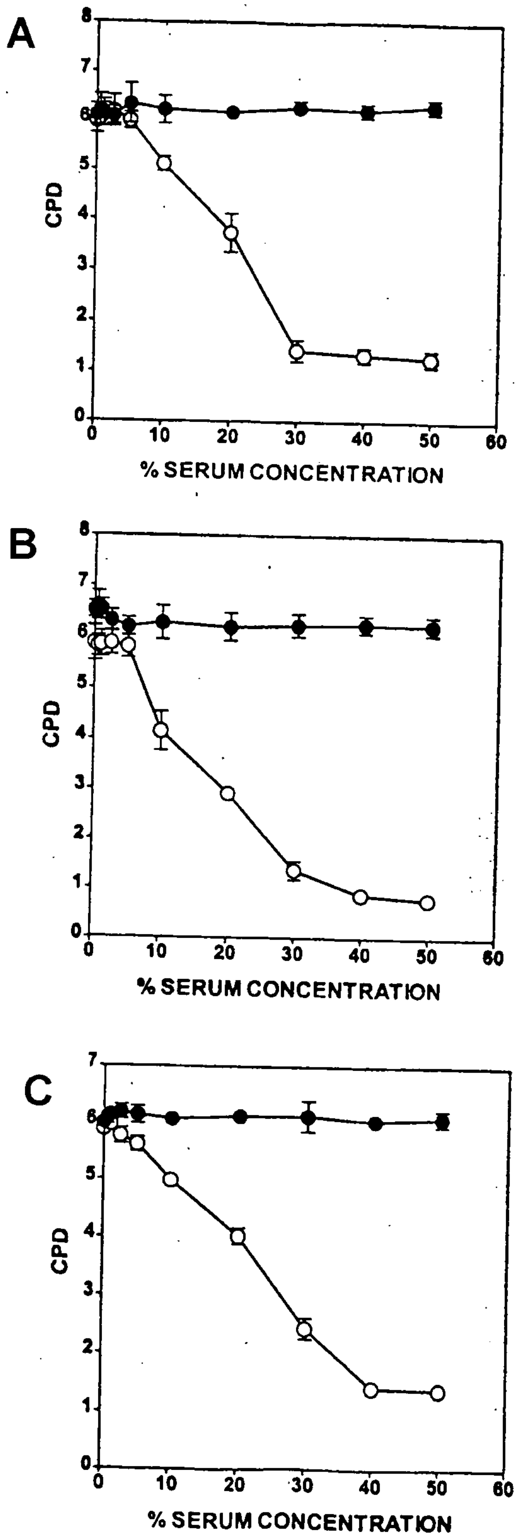
LEGEND:

Closed circles = + E_2

Open circles = − E_2

FIGURE 85

EFFECT OF CDE-SERUM ON ESTROGEN RESPONSIVE GROWTH OF THREE ER$^+$ CANCER CELL LINES IN SFM



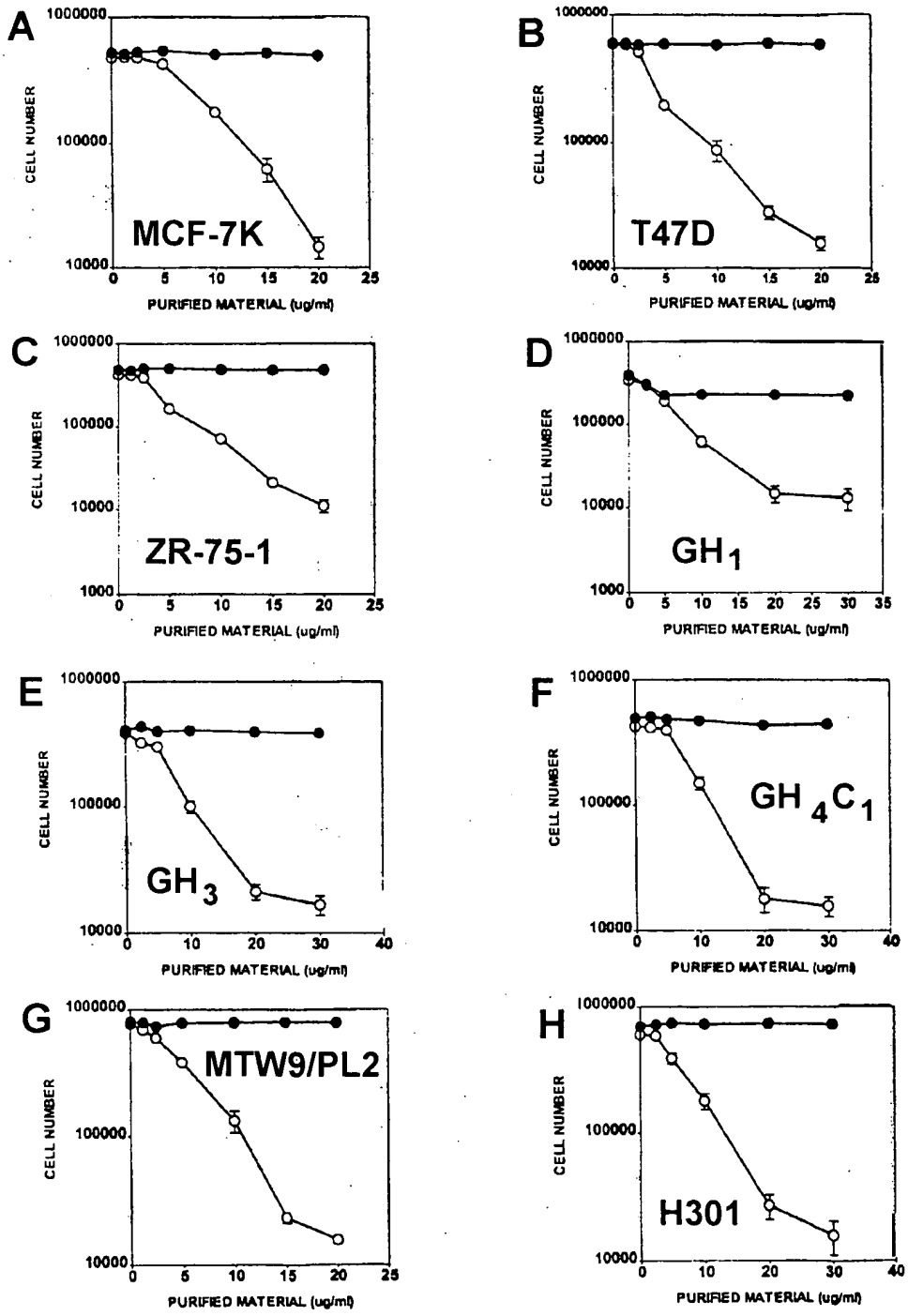
A = T47D IN DDM-2MF

B = MTW9/PL2 IN DDM-2A

C = GH_4C_1 IN PCM 9

FIGURE 86

EFFECT OF CA-PS-POOL II ON ESTROGEN RESPONSIVE GROWTH IN SERUM FREE MEDIUM



LEGEND: Open circles = $- E_2$
Closed circles = $+ E_2$

FIGURE 87

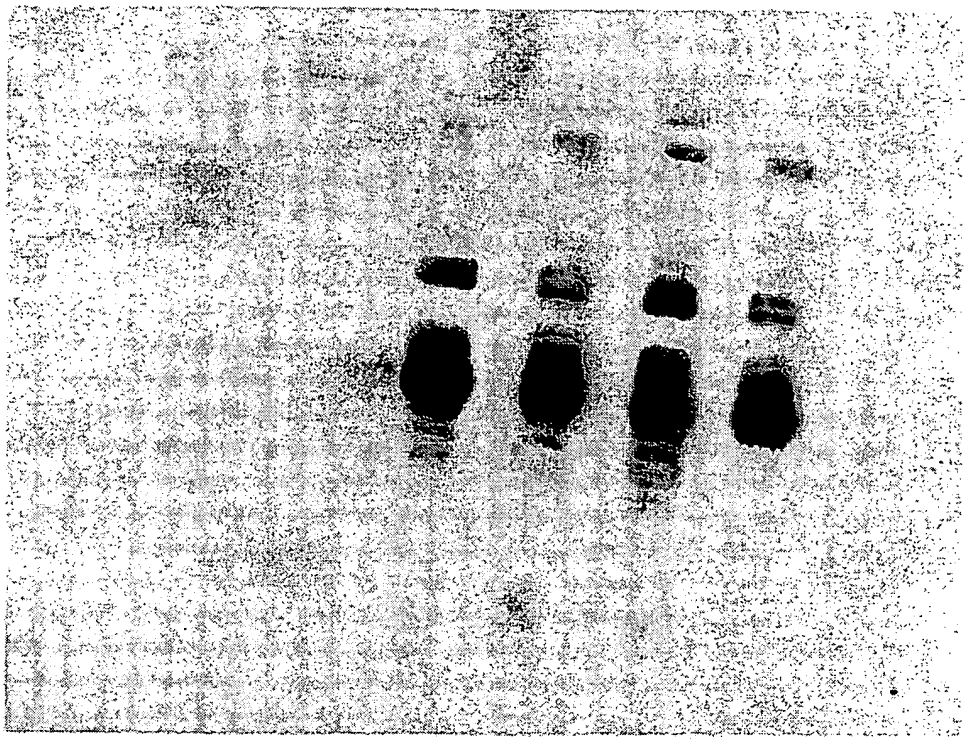
AMINO ACID SEQUENCING - HORSE SHBG

```
                10        20        30        40        50        60        70        80        90       100
hm SHBG  LRPVLPTQSAHDPPAVHLSNGPGQEPIAVMTFDLTKITKTSSSFEVRTWDPEGVIFYGDTNPKDDWFMLGLRDGRPEIQLHNHWAQLTVGAGPRLDDGRW
rb SHBG        TQRAQDSPAVHLINGLGQEPIQVLTFDLTRIVKASSSFELRTWDSEGVIFYGDTNPKDDWFMLGLRDGRPEIQLHNLWAQLTVGAGPRLDDGSW
rt ABP   LRHIDPIQSAQDSPAKYLSNGPGQEPVTVLTIDLTKISKPSSSFEFRTWDPEGVIFYGDTNTEDDWFMLGLRDGQLEIQLHNLWARLTVGFGPRLNDGRW
hs ABP   ...................NGPGQEPVAVMTIDLTQMSKPYSSFEFRTLDPEGVIFYGDTNTKDDWFMLGLRDGQLEIQMHNPWAQLTVGFGPRLNDGRW
                     * * *        * ** *                 ****
               #40:IPGVILVK #25:VVSVLPIQv          #31:IEGVIPIPSV

               110       120       130       140       150       160       170       180       190       200
hm SHBG  HQVEVKMEGDSVLLEVDGEEVLRLRQVSGPLTSKRHPIMRIALGGLLFPASNLRLPLVPALDGCLRRDSWLDKQAEISASAPTSLRSCDVESNPGIFLPP
rb SHBG  HQVHVKLRGDSVLLEVDGKEVLRLSQVSGTLHDKPQPVMKIAVGGLLFPPSSLRLPLVPALDGCLRRGSWLDPQAQLSASAHLSLRSCDVELQPGLFFPP
rt ABP   HPVELKMNGDSLLLWVDGKEMLCLRQVSASLADHPQLSMRIALGGLLLPTSKLRFPLVPALDGCIRRDIWLGHQAQLSTSARTSLGNCDVDLQPGLFFPP
hs ABP   HQVELKMSGDSLQLWVDGKELLCLRQISGTLANNSWPSMRIALGGLLLPTSSLRFPLVPALDGCLRRDTWLGHQVHLSPSAP.NLGNCDVDLQPGLFFPQ
               **              *  **  *         *  **  *           *   *  *         ***   *          * *** *
             #22:SLVYVTNVAK  #26:VVVILAIVPK #34:SVPGLVSPSQ    #37:ATVV?LISDF #20:VQLSPse       #34:SVPGLVSPS
                                                                          #10:VAQFLSTYVIT

               210       220       230       240       250       260       270       280       290       300
hm SHBG  GTQAEFNLRDIPQPHAEPWAFSLDLGLKQAAGSGHLLALGTPENPSWLSLHLQDQKVVLSSGSGPGLDLPLVLGLPLQLKLSMSRVVLSQGSKMKALALP
rb SHBG  GTHAEFSLQDIPQPQTEPWAFSLDLELKPSEGSGRLLALGTPEDPNWLSLHLQDQKVVLSSGMEPGLDLPLAWGLPLQLKLGVSTAVLSQGSKKKALGLP
rt ABP   GTHAEFSLQDIPQPHTDPWTFSLELGFKLVDGAGRLLTLGTGTNSSWLTLHLQDQTVVLSSEAEPKLALPLAVGLPLQLKLDVFKVALSQGPKMEVLSTS
hs ABP   GTHAEFSLQDIPQPRTDPWSFSLELGLKLVDGSGCLLALGTRTNSSWLSLHLQDQKVVLSSGVEPKLVLALDMGLPLQLKLDILKVVLSQGPKTEVLGAS
             * *  **                                            **      ****
         Q                                               #26:VVVILAIVPK
         #41:VFALAPIPGVLK                                           #9:LAVQVR

               310       320       330       340       350       360       370
hm SHBG  PLGLAPLLNLWAKPQGRLFLGALPGEDSSTSFCLNGLWAQGQRLDVDQALNRSHEIWTHSCPQSPGNGTDASH
rb SHBG  SPGLGPLLNLWAKPQGRLFLGALPGEDSSASFCLDGLWTVGQKLDMDKALNRSHDIWTHSCPQSPGNGTDASH
rt ABP   LLRLASLWRLWSHPQGRLSLGALPGEDSSASFCLSDLWVQGQRLDIDKALSRSQDIWTHSCPQSPSNDTHTSH
hs ABP   ASRLAALRTLWSHPQGLLSLGALAGDNSSASFCLSDLWVQGQRLDIDQALNRSQNIWTHSCPHSPNNVSHISH
```

FIGURE 88

WESTERN ANALYSIS OF CBG (POOL I) AND

SHBG (POOL II) PREPARATION WITH ANTI-54 kDa



1 = CBG PREPARATION #5

2 = CBG PREPARATION #6

3 = SHBG PREPARATION #5.1

4 = SHBG PREPARATION #5.2

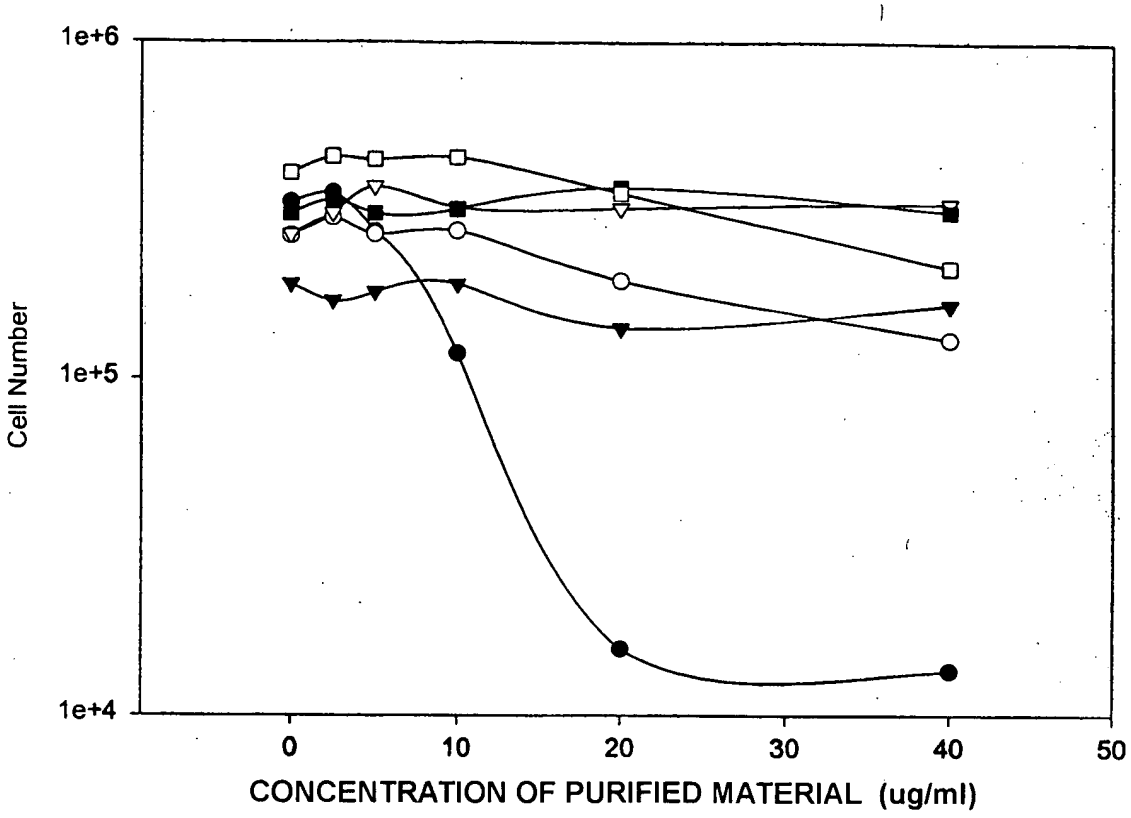
5 = SHBG PREPARATION #6.1

6 = SHBG PREPARATION #6.2

ANTIBODY = RABBIT ANTI-54 kDa 1:5000 DILUTION

FIGURE 89

EFFECT OF ANTI-54kDa ANTISERUM ON MTW9/PL2 CELLS GROWN IN THE PRESENCE OF CA-PS-POOL II



LEGEND:

- No antibody
- Antibody 1:5000
- Antibody 1:1000
- Antibody 1:500
- Antibody 1:100
- Antibody 1:50

FIGURE 90

WESTERN BLOT OF COMMERCIAL PREPARATIONS OF HORSE IgA, IgG AND IgM WITH THE ANTI-54 kDa ANTIBODY

FIGURE 91

EFFECT OF COMMERCIALLY PURIFIED HORSE IgG ON MTW9/PL2 CELL GROWTH IN 2.5% CDE-HORSE SERUM

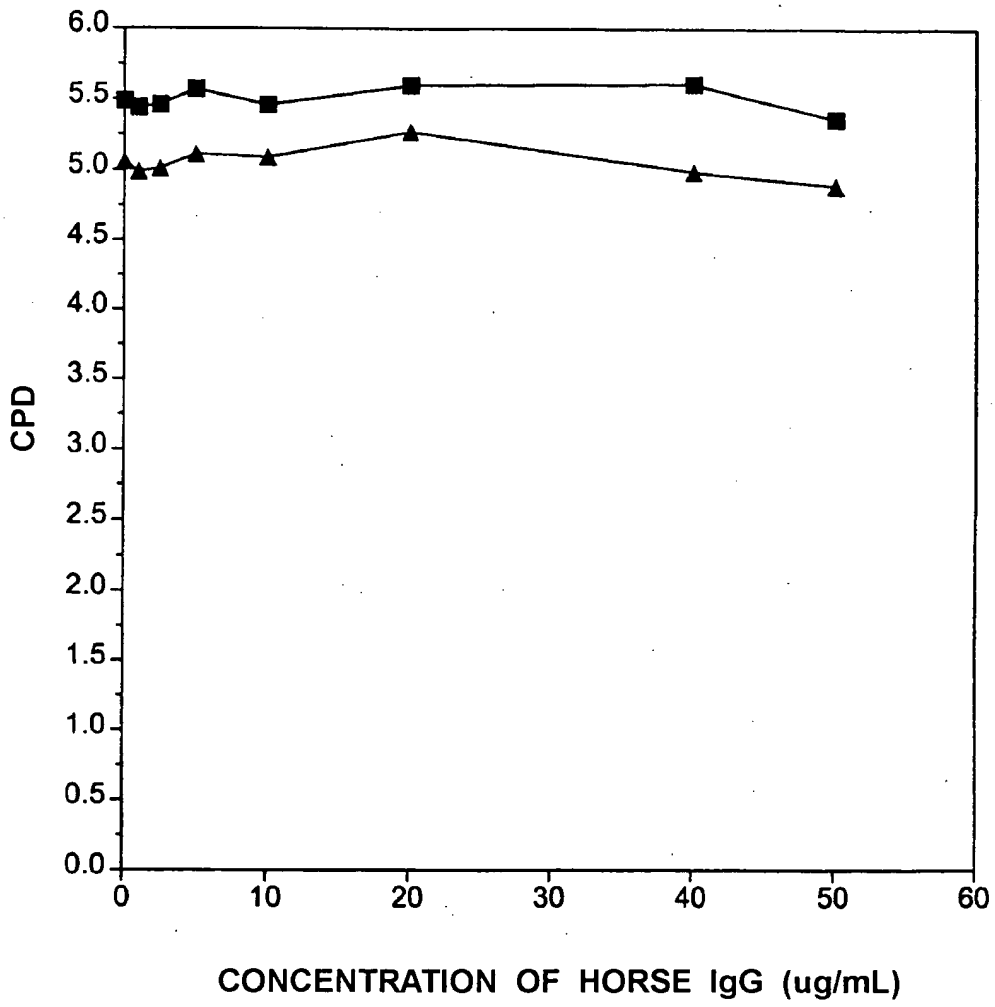
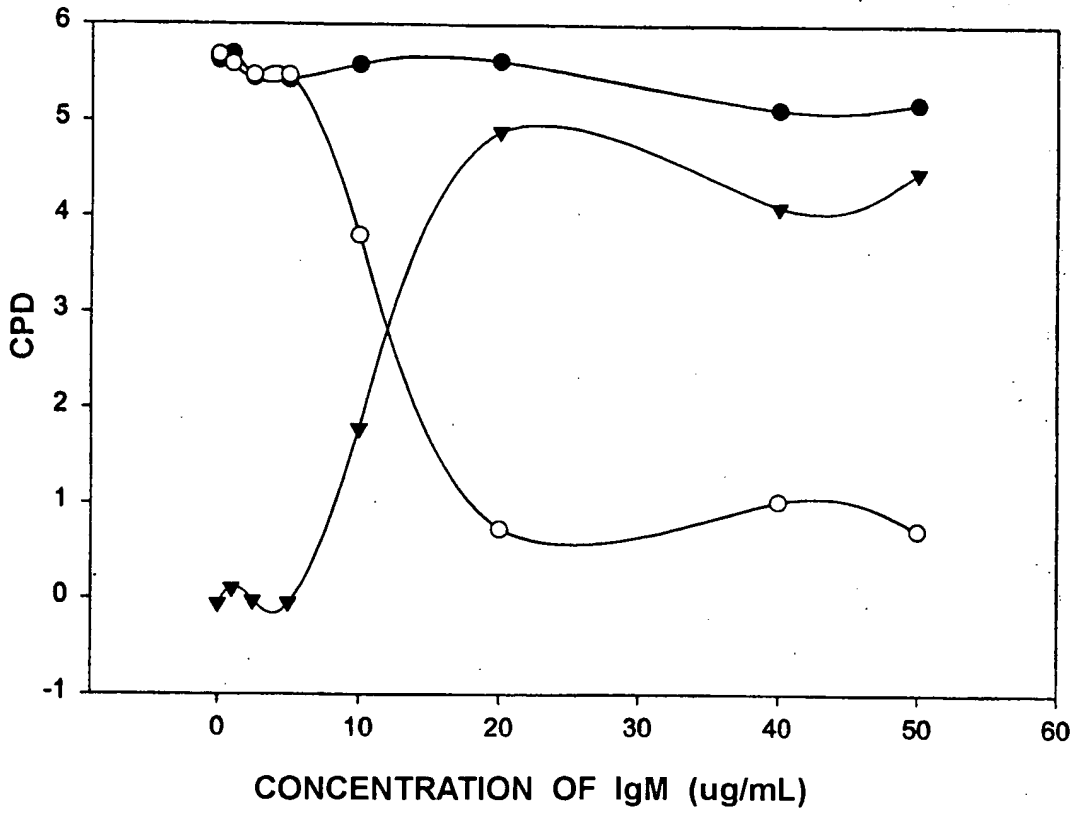


FIGURE 92

EFFECT OF HORSE IgM ON GROWTH OF THE MTW9/PL2 CELLS IN 2.5% CDE HORSE SERUM $\pm E_2$



LEGEND:

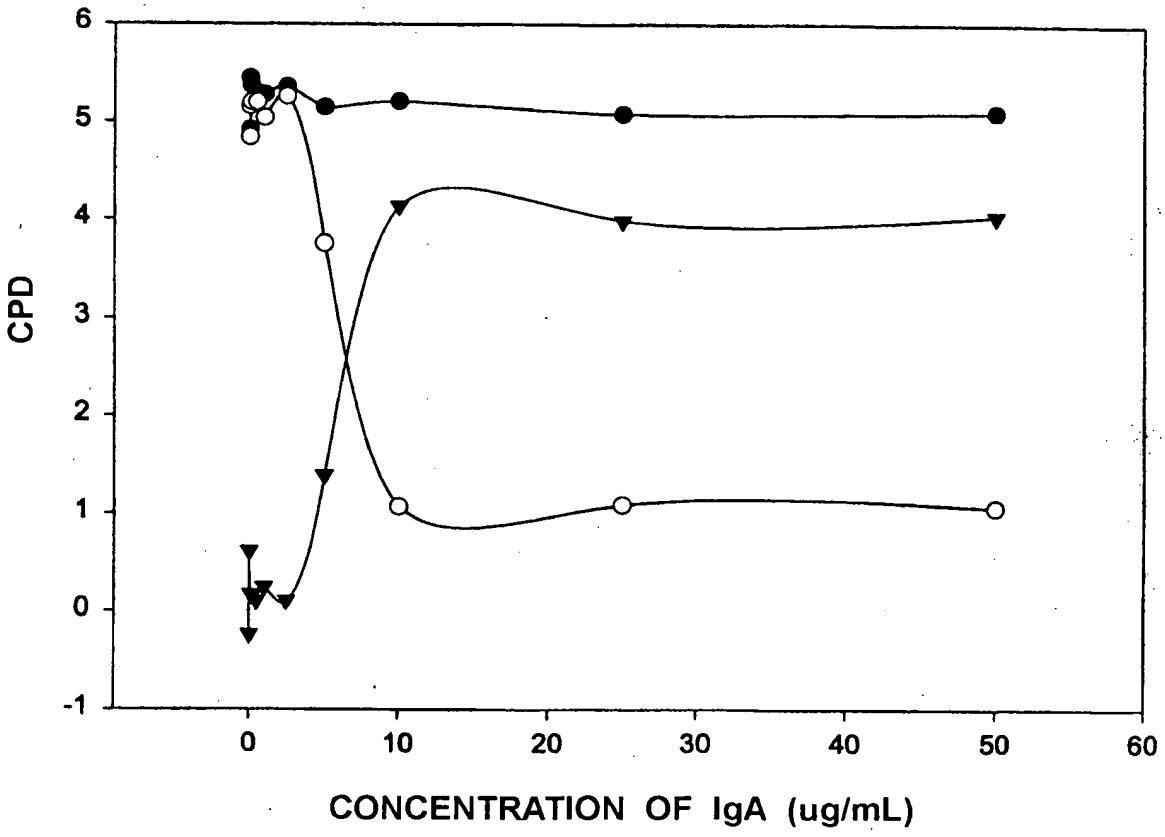
● = + E_2

○ = − E_2

▼ = Estrogenic effect

FIGURE 93

EFFECT OF HORSE IgA ON GROWTH OF THE MTW9/PL2 CELLS IN 2.5% CDE HORSE SERUM ± E_2



LEGEND:

—●— = + E_2

—○— = − E_2

—▼— = Estrogenic effect

FIGURE 94

SDS PAGE AND WESTERN ANALYSIS OF RAT "SHBG-LIKE" PREPARATIONS

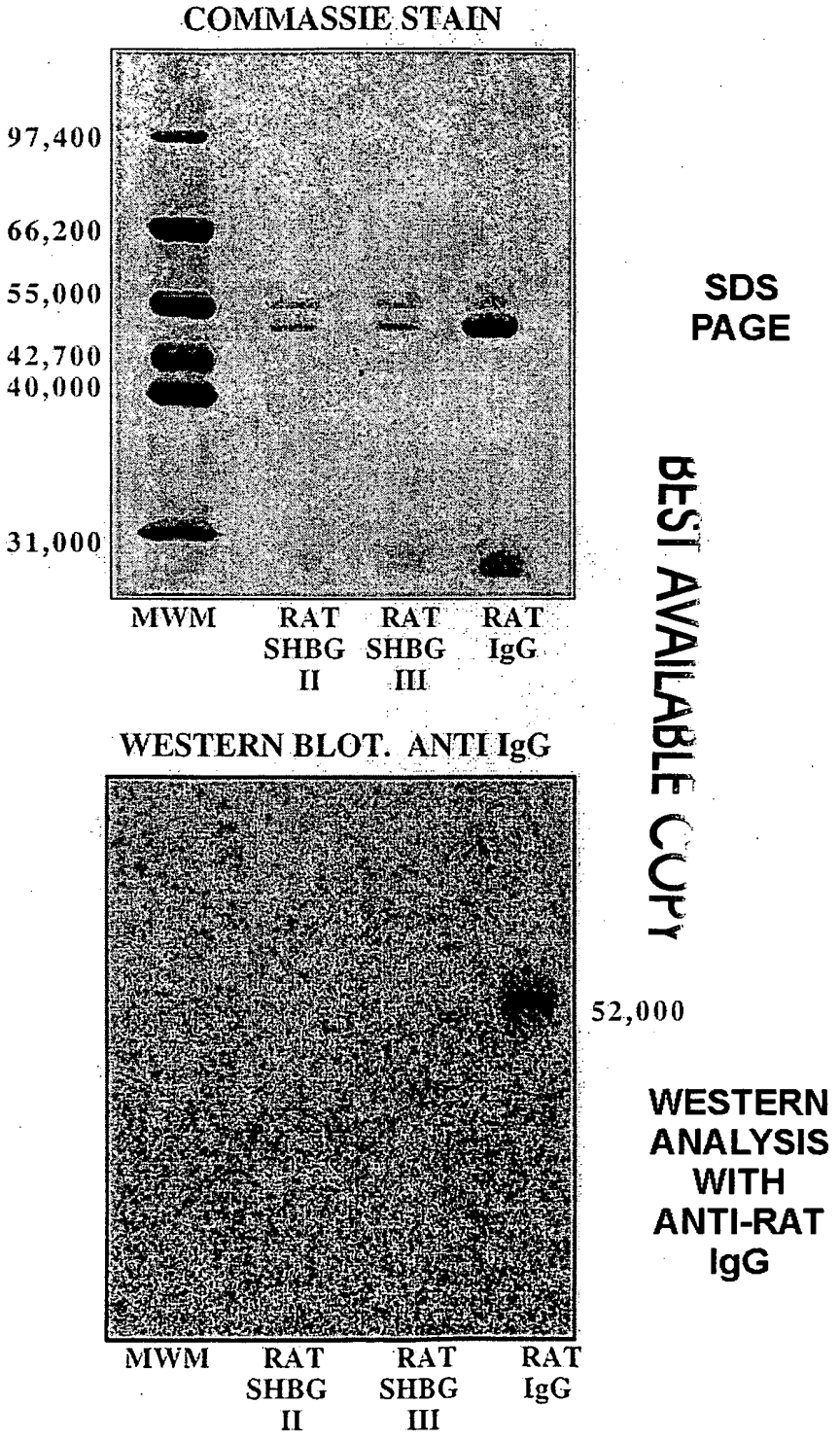


FIGURE 95

CROSSREACTION OF THE PURIFIED RAT "SHBG-LIKE" PROTEINS WITH ANTI- IgA, IgG1 AND IgM MONOCLONAL ANTIBODIES

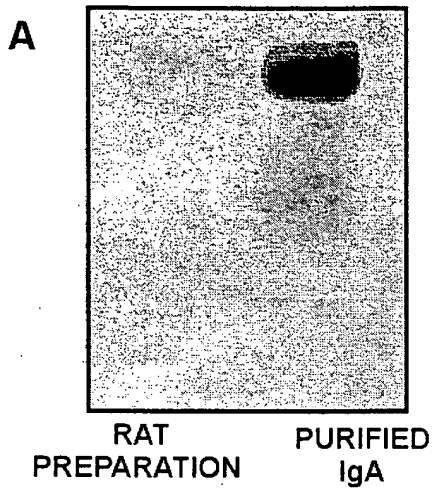


FIGURE 96

AMINO ACID SEQUENCING - RAT SHBG

```
                10        20        30        40        50        60        70        80        90       100
hmSHBG  ..PVL.T...H.P..VH.........IA.M.F.....T.T.....V................PK...........RP......H..Q....A....D....
rbSHBG      T.R......VH.I..L....IQ...F...R.V.A......L....S..........PK...........RP.........Q....A....D..S.
hsABP                  ........A.M.....QM...Y........L.............K...............M..P..Q.............
rtABP   LRHIDPIQSAQDSPAKYLSNGPGQEPVTVLTIDLTKISKPSSSFEFRTWDPEGVIFYGDTNTEDDWFMLGLRDGQLEIQLHNLWARLTVGFGPRLNDGRW
                             **  ****        *   * * **         ***                        **    *      **
                        #37':GPFVTPVTVTK  #9:IEQY?STFK    #29:IFYPI?IYTQ            #21:EVQLVEIGGGLVQPGR
                                 P V          LP              GL  VD VE
                                              E  #37:DYFR

               110       120       130       140       150       160       170       180       190       200
hmSHBG  .Q..V..E...V..E...E.V.R.....GP.TSKRHPI.........F.A.N..L.........L...S..DK..EI.A..P...RS...ESN..I.L..
rbSHBG  .Q.HV.LR...V..E.....V.R.S...GT.H.K..PV.K..V....F.P.S..L.........L..GS..DP.....A..HL..RS...E.........
hsABP   .Q.....S....Q........L.....I.GT..NNSWP..............S...........L...T.....VH..P..PN-...............Q
rtABP   HPVELKMNGDSLLLWVDGKEMLCLRQVSASLADHPQLSMRIALGGLLLPTSKLRFPLVPALDGCIRRDIWLGHQAQLSTSARTSLGNCDVDLQPGLFFPP
                                                                                                    ***  *
                                                                                           #18:VVSGLFPVP
                                                                                                EAPIA
                                                                                                T

               210       220       230       240       250       260       270       280       290       300
hmSHBG  ..Q...N.R......AE..A...D..L.QAA.S.H..A...PE.P...S......K.....GSG.G.D...VL.........SMSR.V....S..KA.ALP
rbSHBG  ..............Q.E..A...D.EL.PSE.S.....A...PEDPN..S......K.....GM..G.D....W.........G.STAV....S.KKA.GLP
hsABP   ..............R....S......L.....S.C..A...R......S......K.....GV....V.A.DM..........IL..V......T...GA.
rtABP   GTHAEFSLQDIPQPHTDPWTFSLELGFKLVDGAGRLLTLGTGTNSSWLTLHLQDQTVVLSSEAEPKLALPLAVGLPLQLKLDVFKVALSQGPKMEVLSTS
           *                        ** **  **  *
         ISGAFIAF          #18:VVSGLFPVPISGAFIAF
         K  P V                EAPIA  K  P V
                               T

               310       320       330       340       350       360       370
hmSHBG  P.G..P.LN..AK.....F.........T....NG..A......V.Q..N..HE..........G.G.DA..
rbSHBG  SPG.GP.LN..AK.....F...............DG..TV..K..M....N..H............G.G.DA..
hsABP   AS...A.RT.......L.......A.DN...................Q..N...N.......H..N.VS.I..
rtABP   LLRLASLWRLWSHPQGRLSLGALPGEDSSASFCLSDLWVQGQRLDIDKALSRSQDIWTHSCPQSPSNDTHTSH
```

FIGURE 97

SDS PAGE (A) AND WESTERN ANALYSIS (B) WITH ANTI-SHBG AND RAT Ig'S

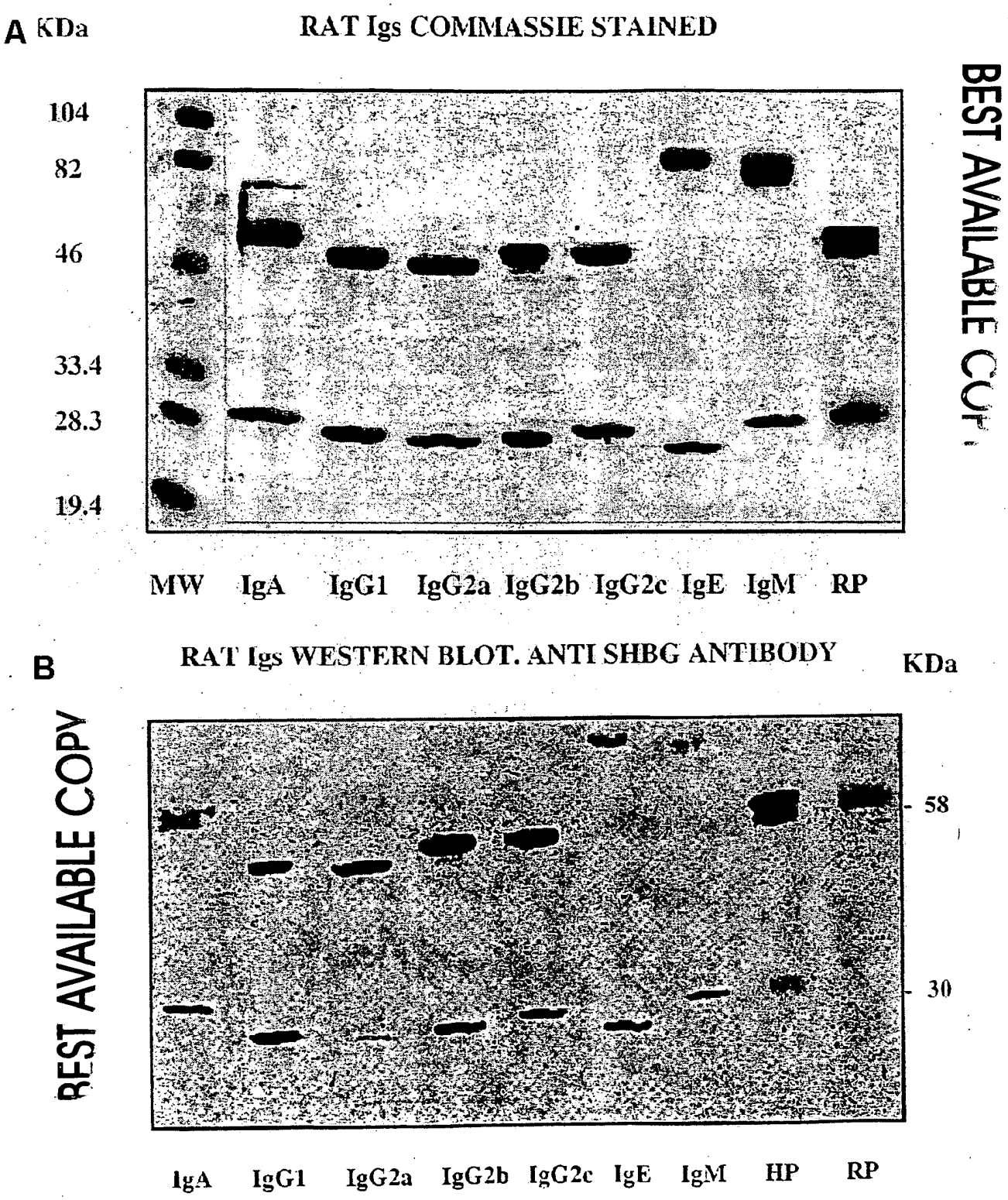
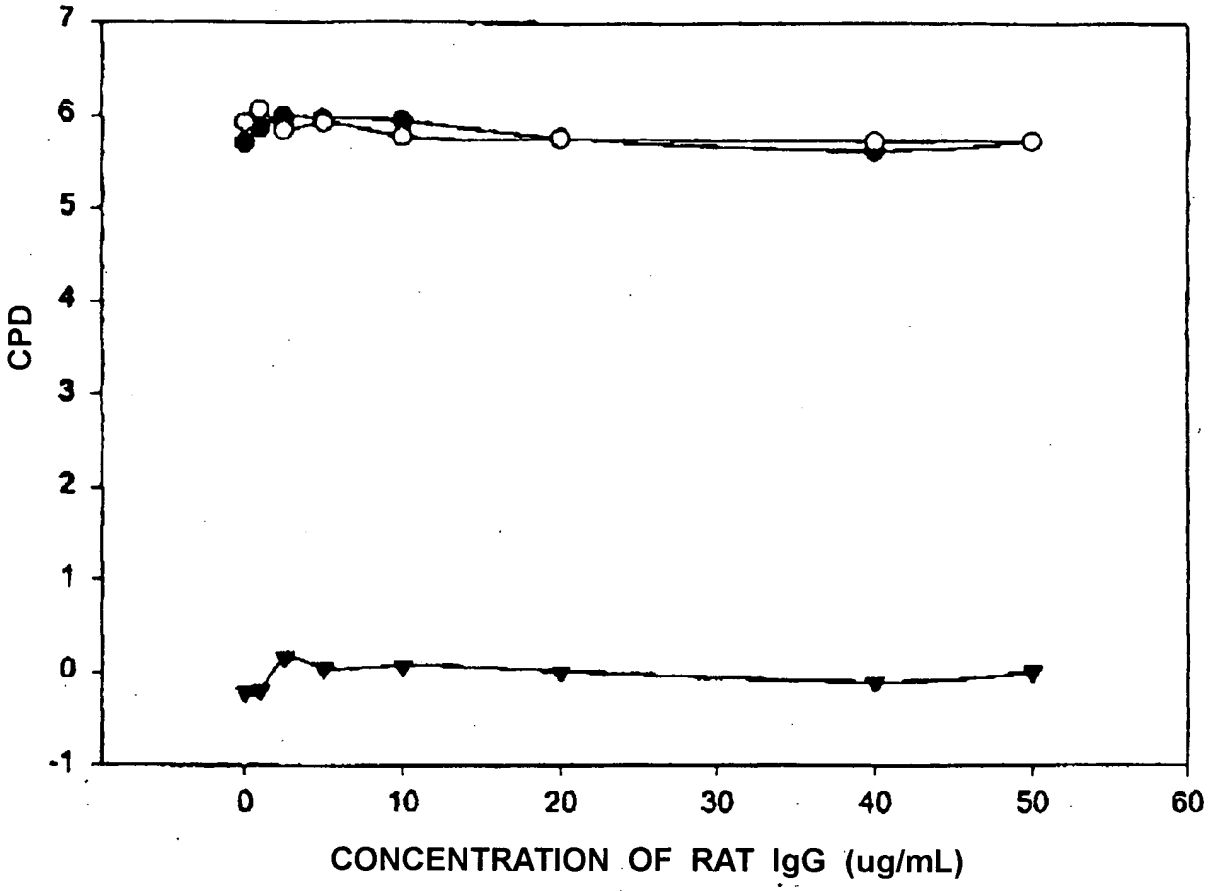


FIGURE 98

EFFECT OF RAT IgG ON MTW9/PL2 CELL GROWTH IN 2.5% CDE RAT SERUM



LEGEND:

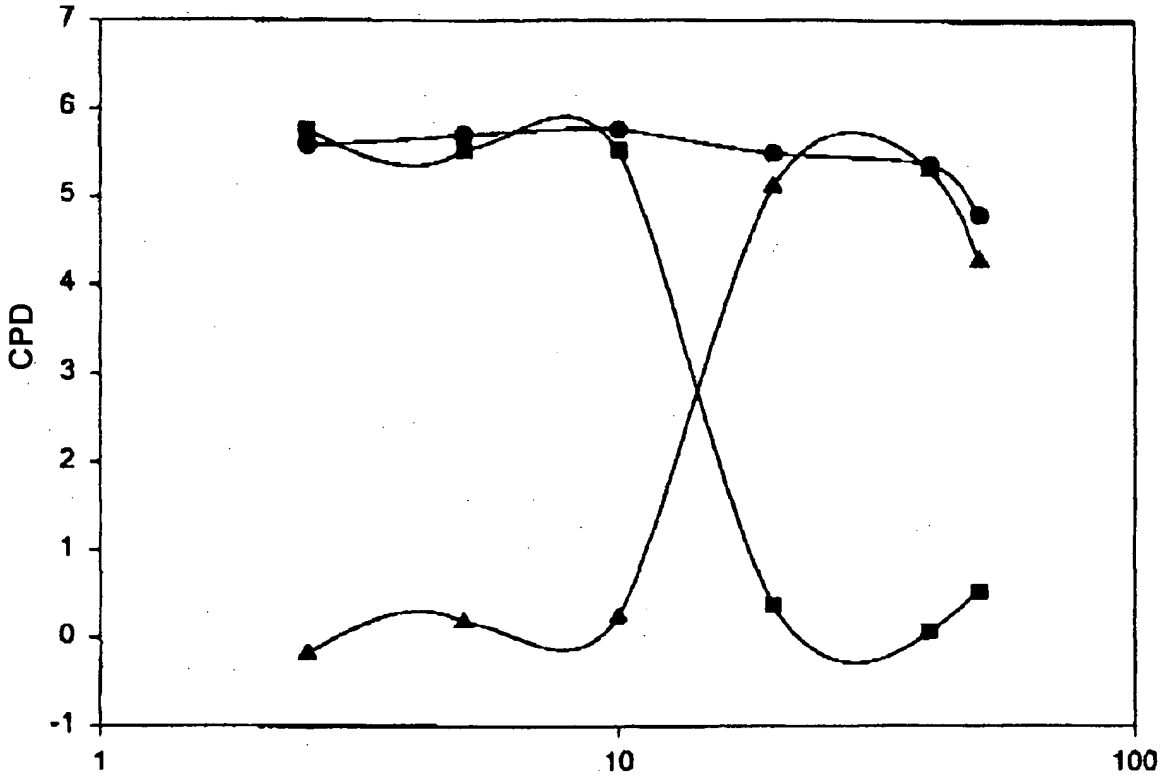
Closed circles = + E_2

Open circles = – E_2

Closed triangles = Estrogenic effect

FIGURE 99

EFFECT OF RAT IgA ON MTW9/PL2 CELL GROWTH IN 2.5% CDE RAT SERUM



LEGEND:

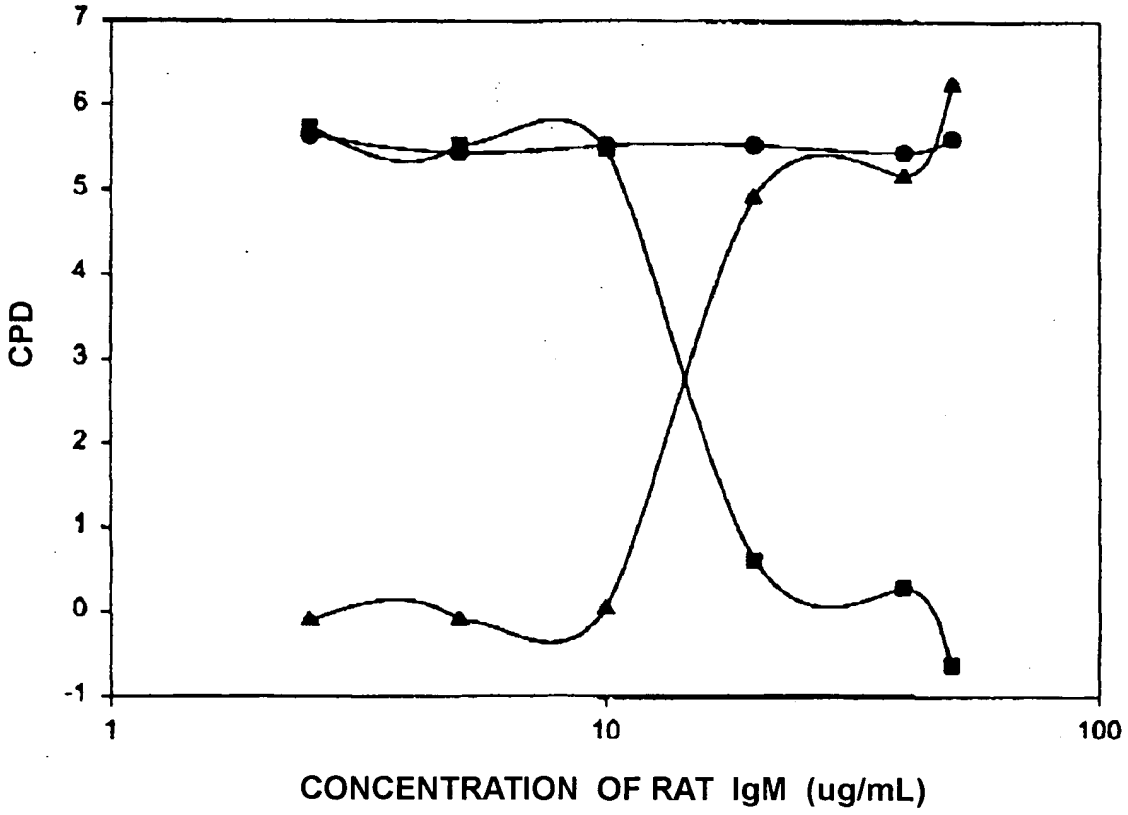
Closed circles = + E_2

Closed squares = − E_2

Closed triangles = Estrogenic effect

FIGURE 100

EFFECT OF RAT IgM ON MTW9/PL2 CELL GROWTH IN 2.5% CDE RAT SERUM



LEGEND:

Closed squares = $- E_2$

Closed circles = $+ E_2$

Closed triangles = Estrogenic effect

FIGURE 101

ELUTION OF IgM FROM MANNAN BINDING PROTEIN COLUMN

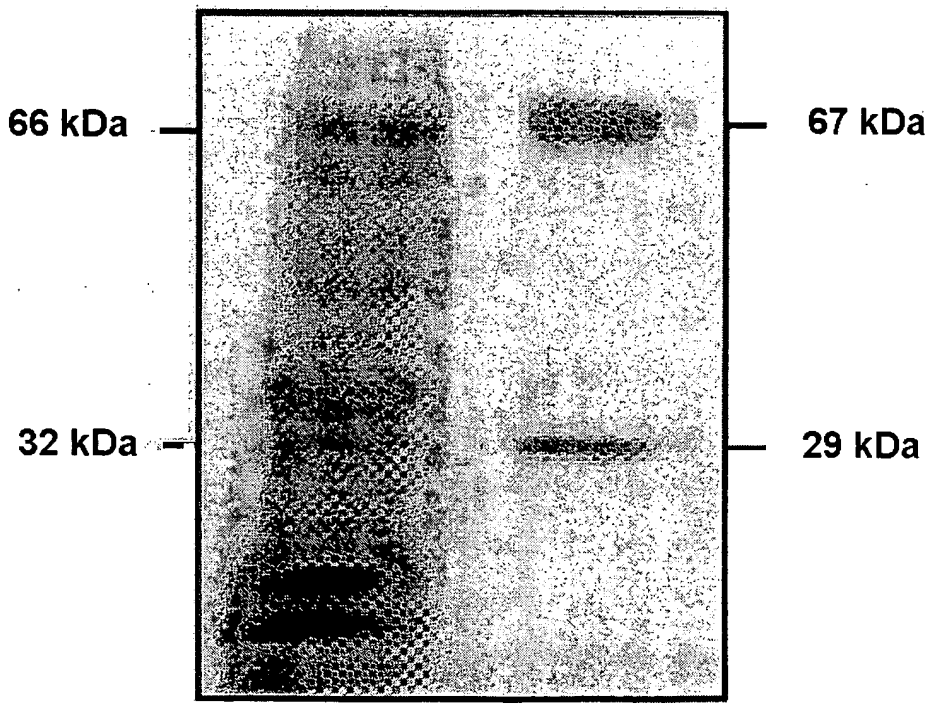
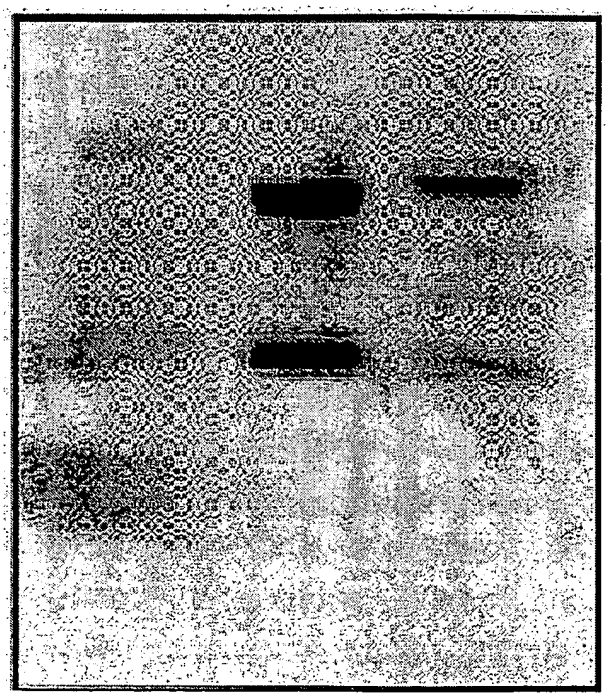


FIGURE 102

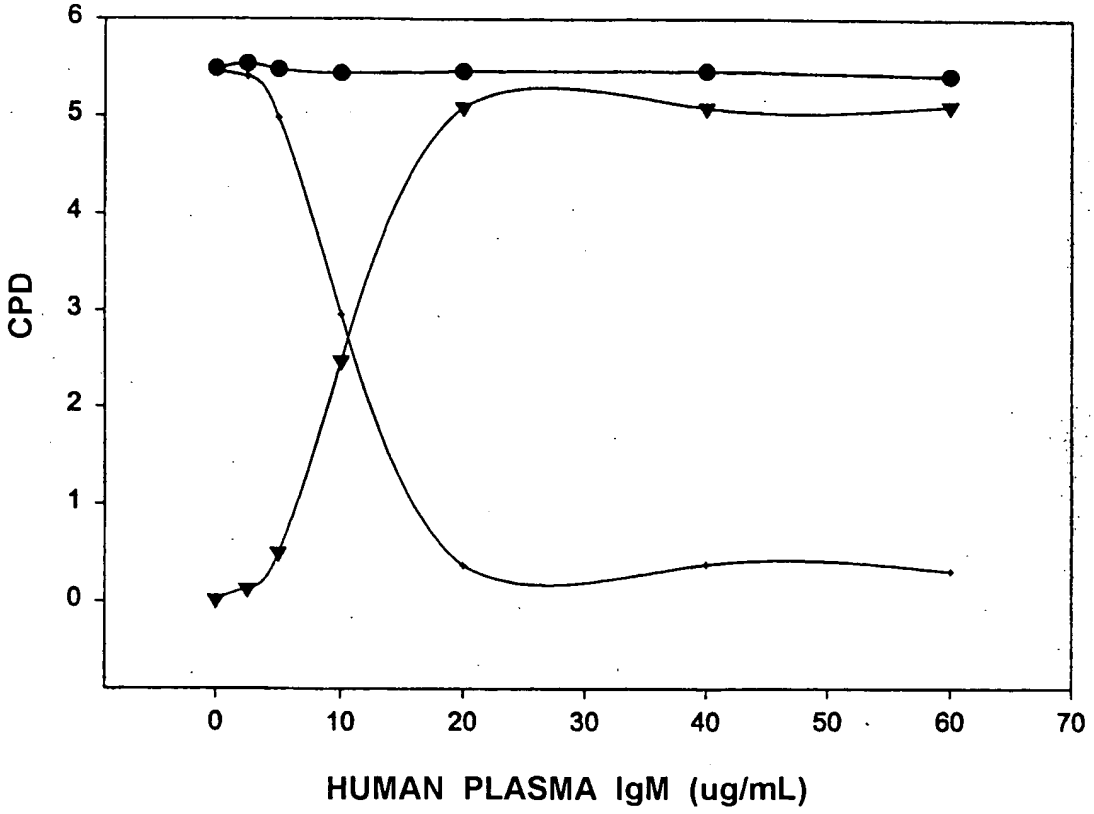
IgM PURIFICATION FROM PLASMA BY JACALIN



MW HUMAN IgA PURIFIED IgA

FIGURE 103

EFFECT OF IgM ISOLATED FROM HUMAN PLASMA ON MTW9/PL2 GROWTH IN SERUM-FREE CONDITIONS



LEGEND:

= + E_2

= − E_2

= Estrogenic effect

FIGURE 104

THE EFFECT OF VARIOUS IgA AND IgM PREPARATIONS ON MTW9/PL2 CELLS GROWN IN SERUM-FREE MEDIUM

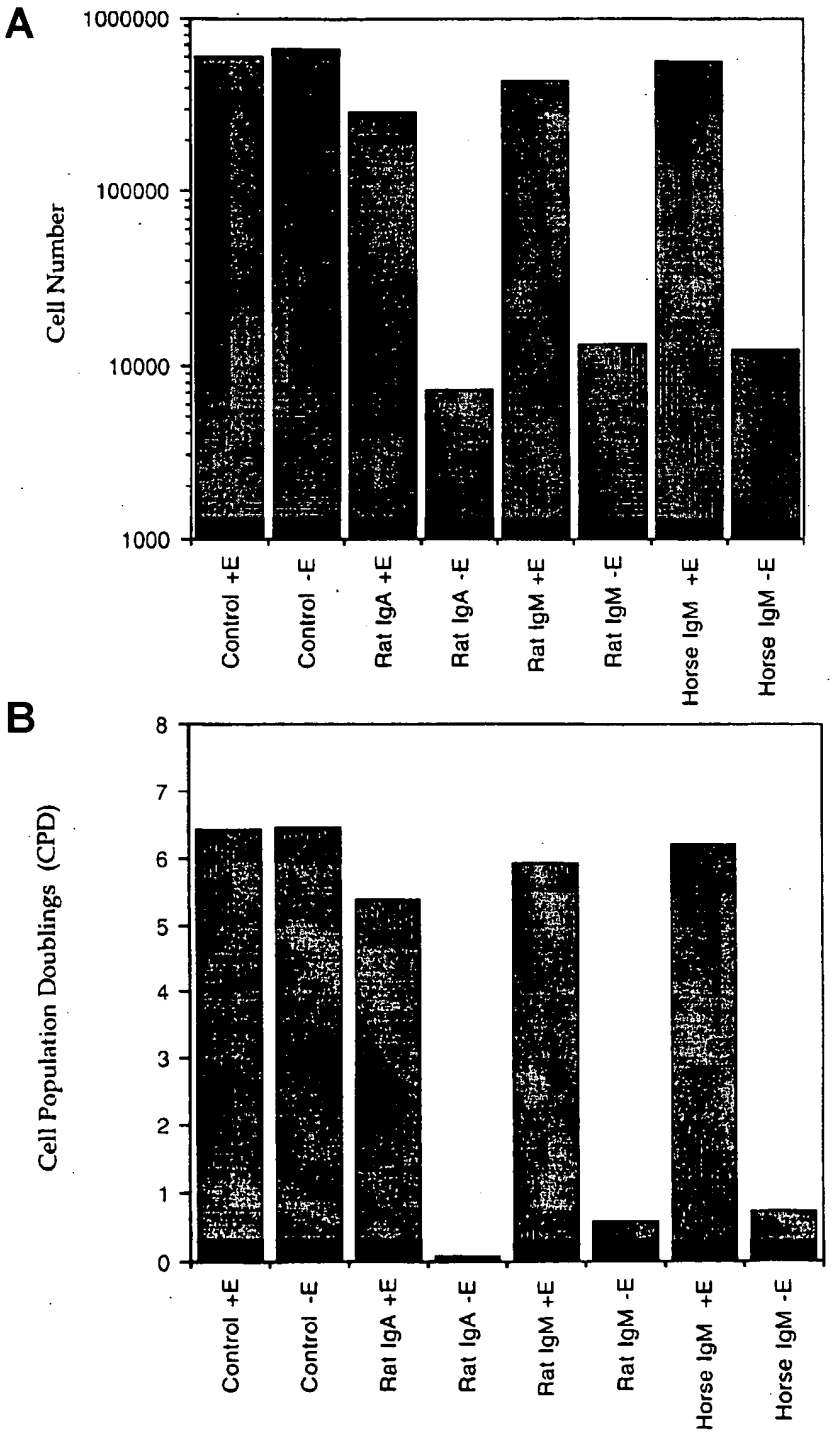
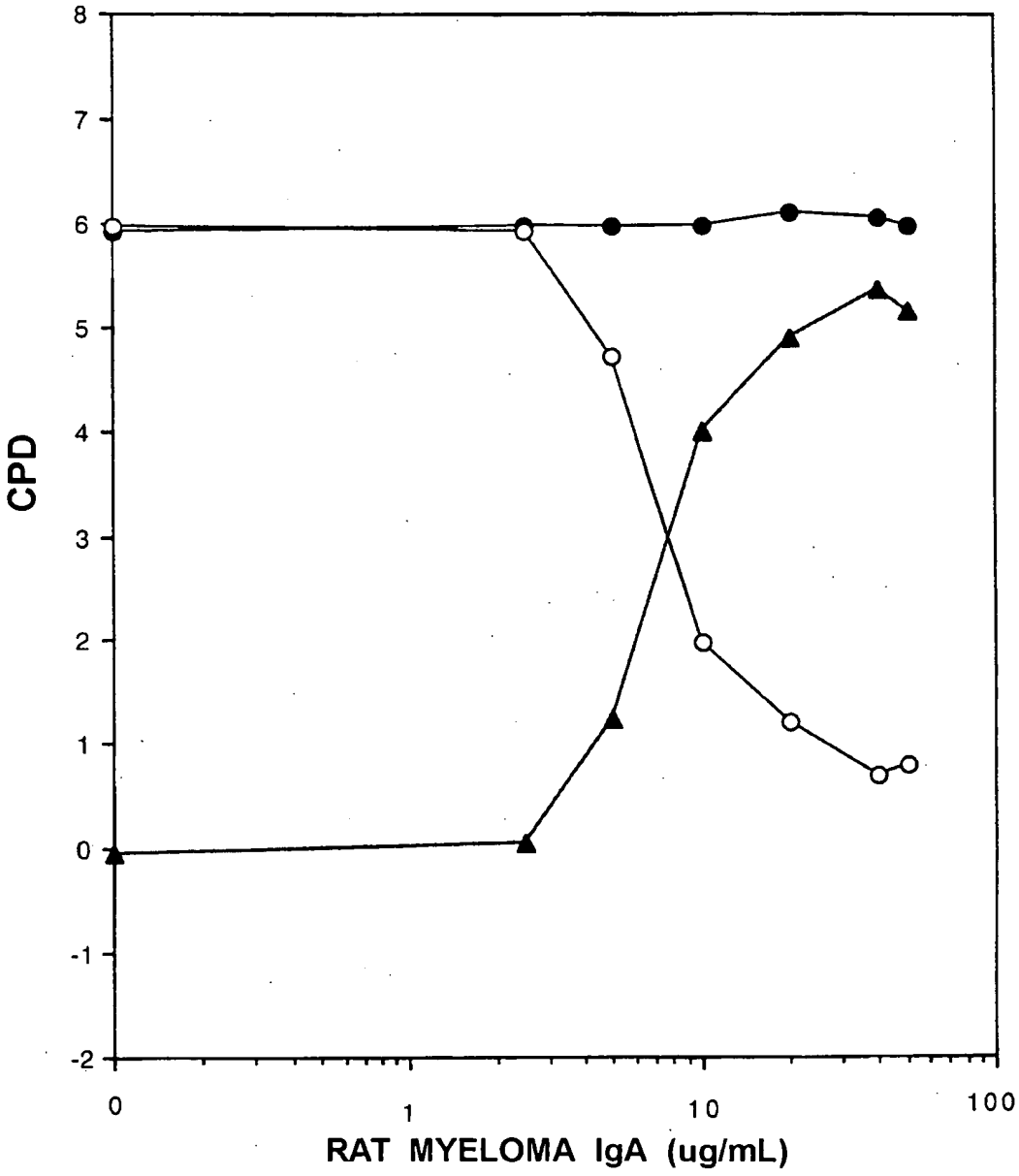


FIGURE 105

RAT MYELOMA IgA TITRATION ON GH_1 CELLS GROWN IN SERUM-FREE CONDITIONS



LEGEND:

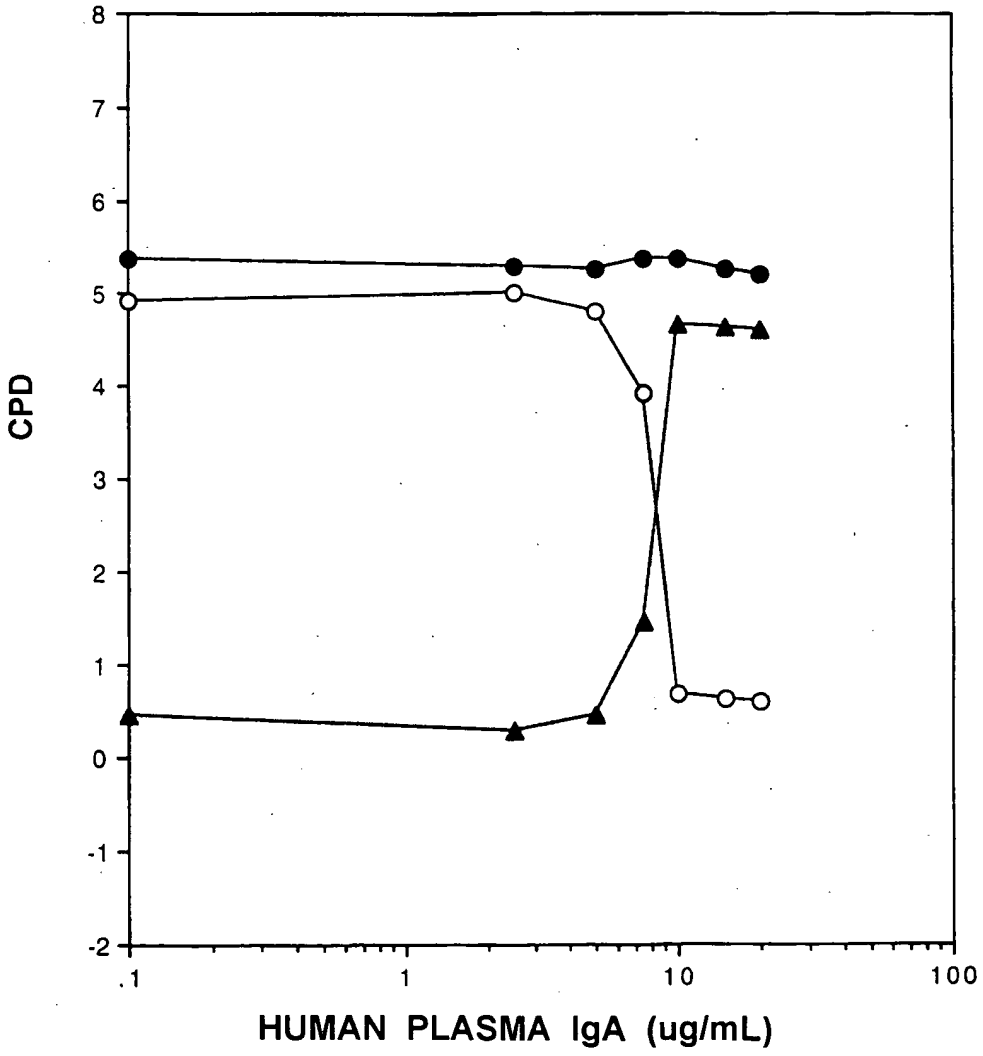
Closed circles = + E_2

Open circles = – E_2

Closed triangles = Estrogenic effect

FIGURE 106

HUMAN PLASMA IgA TITRATION ON GH_1 CELLS GROWN IN SERUM-FREE CONDITIONS



LEGEND:

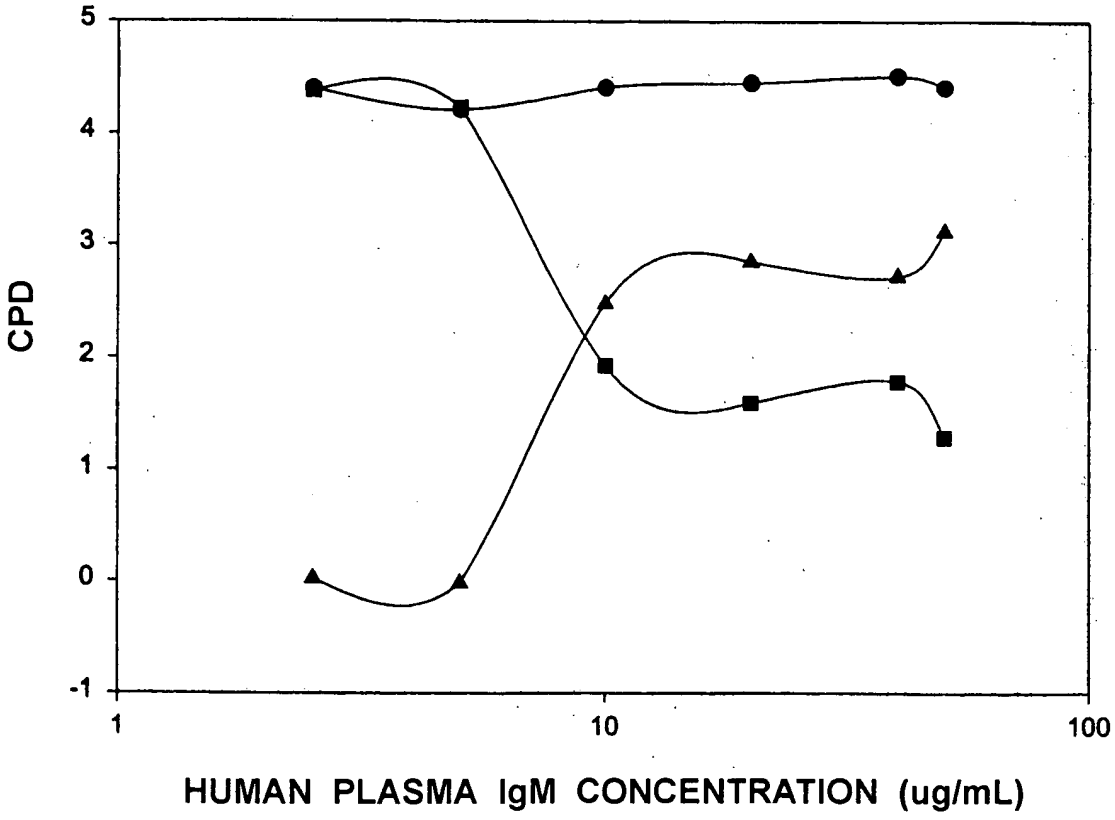
Closed circles = + E_2

Open circles = – E_2

Closed triangles = Estrogenic effect

FIGURE 107

HUMAN PLASMA IgM TITRATION ON GH_1 CELLS GROWN IN SERUM-FREE CONDITIONS



LEGEND:

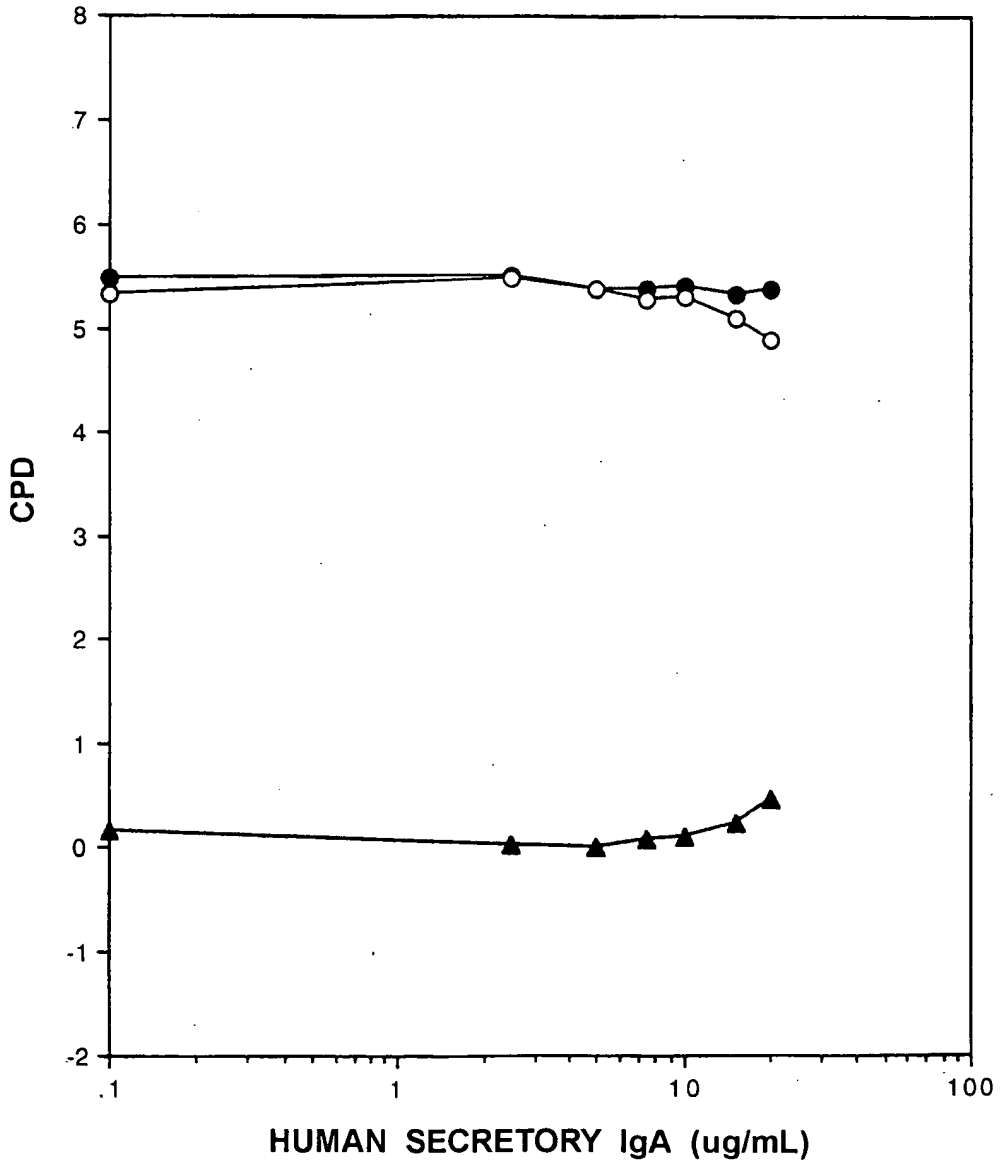
—●— = + E_2

—■— = − E_2

—▲— = Estrogenic effect

FIGURE 108

EFFECT OF HUMAN SECRETORY IgA ON GH_1 CELLS GROWN IN SERUM-FREE CONDITIONS



LEGEND:

Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 109

MECHANISM OF TRANSCYTOSIS OF IgA AND IgM BY MUCOSAL EPITHELIAL CELLS

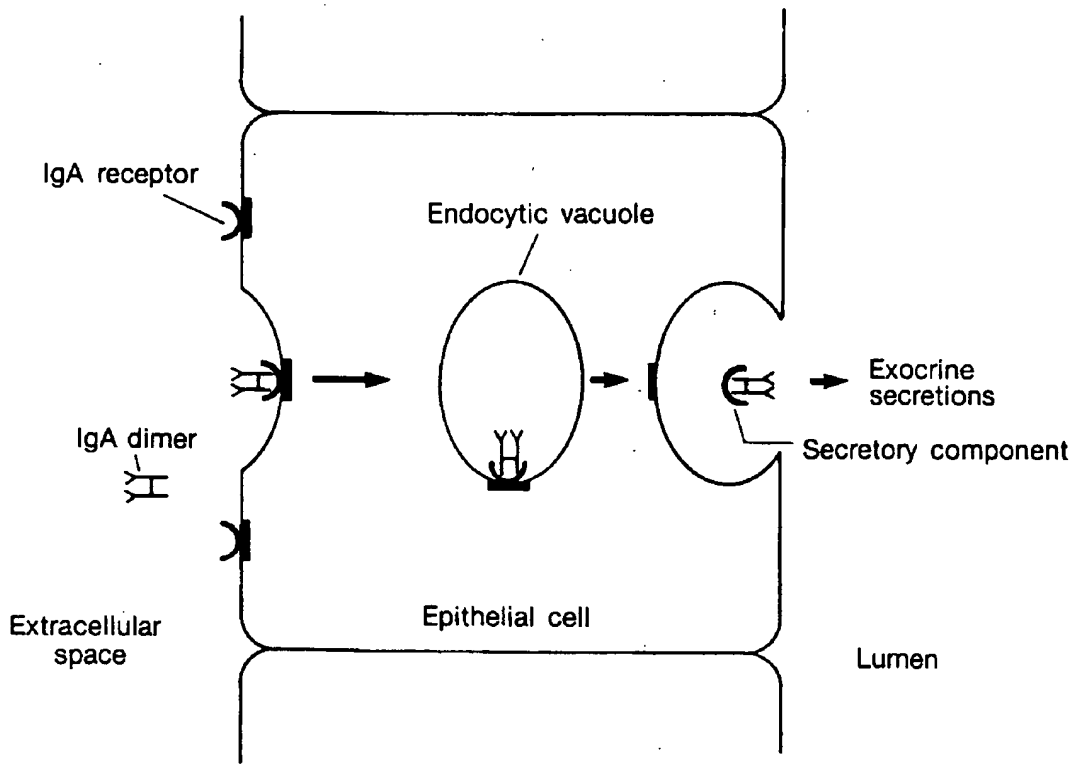
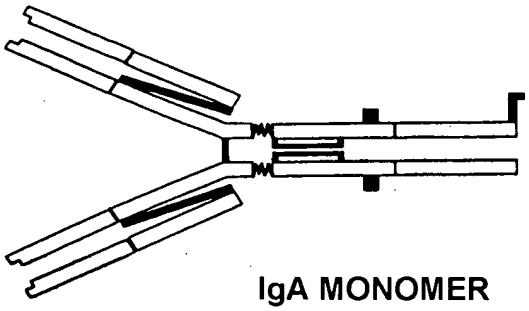


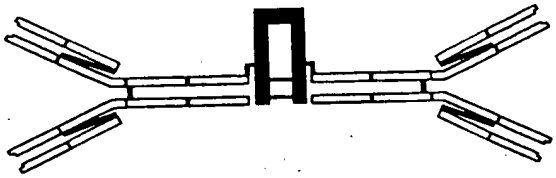
FIGURE 110

ESSENTIAL STRUCTURES OF HUMAN PLASMA AND SECRETORY IgA



IgA MONOMER (INACTIVE)

J CHAIN



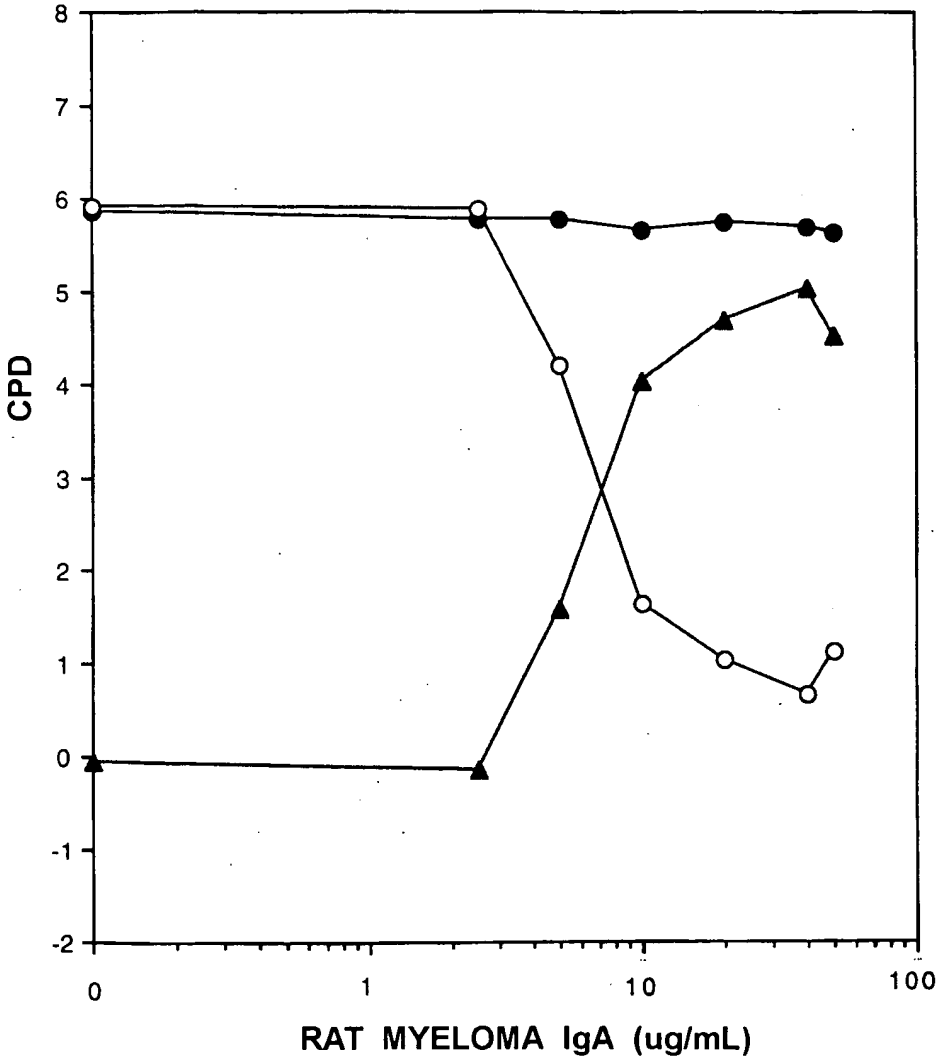
IgA DIMER WITH ATTACHED J CHAIN (ACTIVE)

SECRETORY PIECE OR SECRETORY COMPONENT (80% POLY-IgR)

SECRETORY IgA SHOWING J CHAIN AND SECRETORY COMPONENT (INACTIVE)

FIGURE 111

EFFECT OF RAT MYELOMA IgA ON GH_3 CELLS GROWN IN SERUM-FREE MEDIUM



LEGEND:

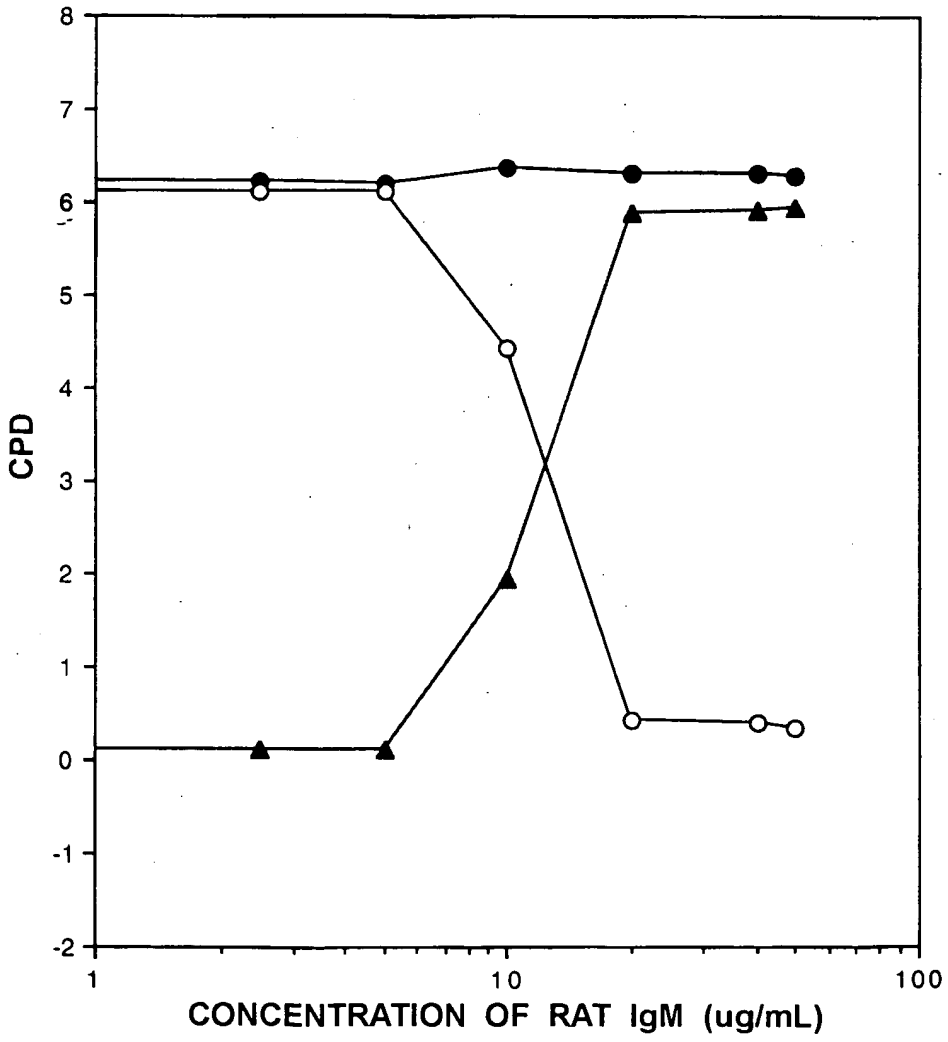
Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 112

EFFECT OF RAT IgM ON GH_3 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

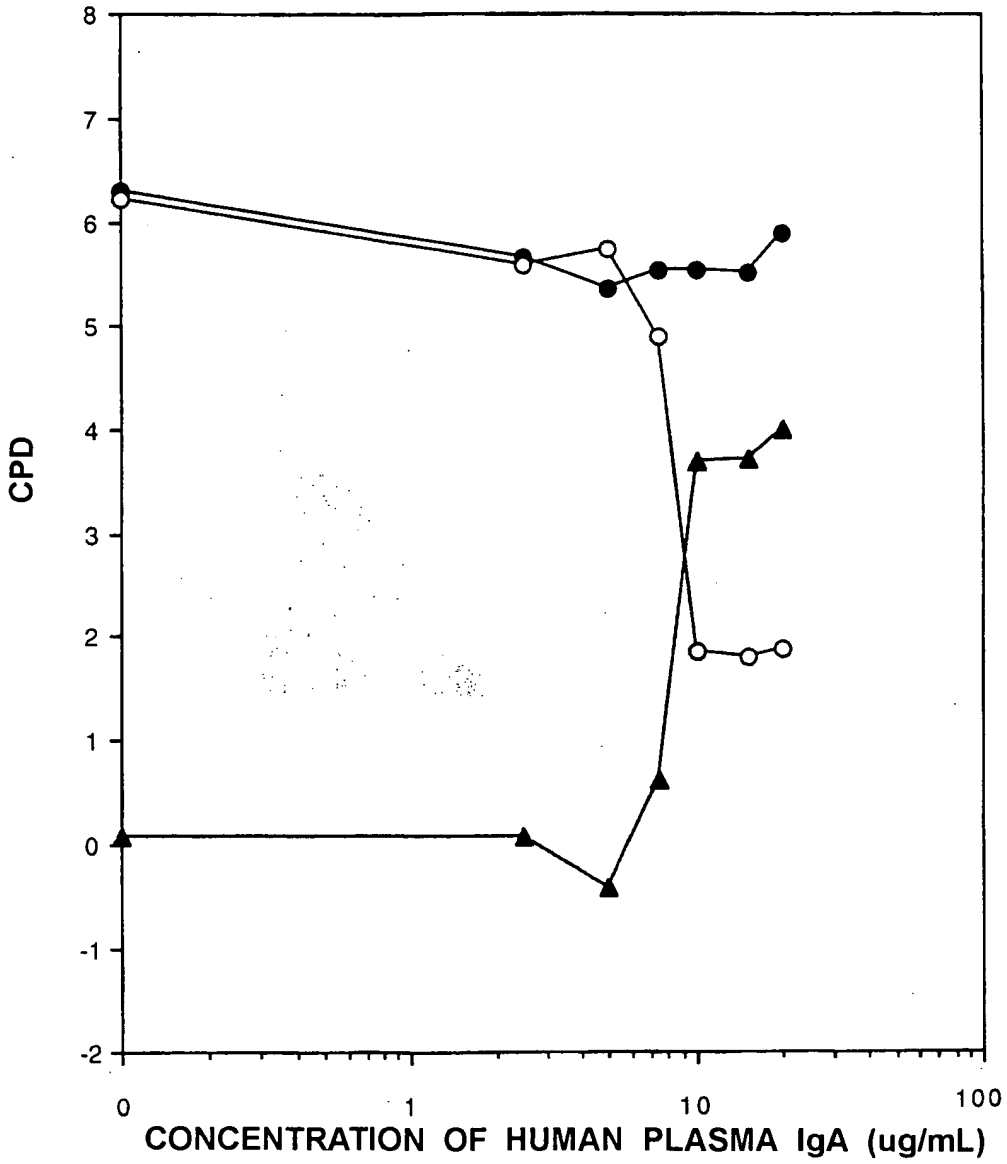
● = + E_2

○ = − E_2

▲ = Estrogenic effect

FIGURE 113

EFFECT OF HUMAN PLASMA IgA ON GH_3 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

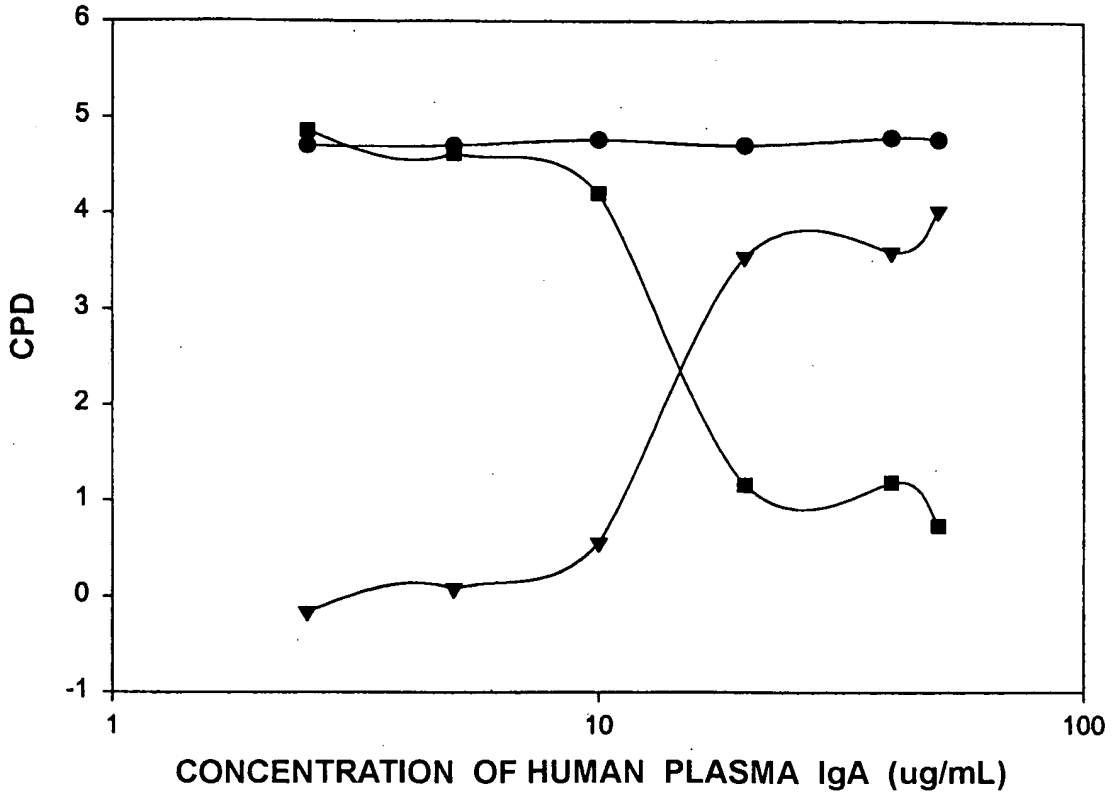
Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 114

EFFECT OF HUMAN PLASMA IgM ON GH $_3$ CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

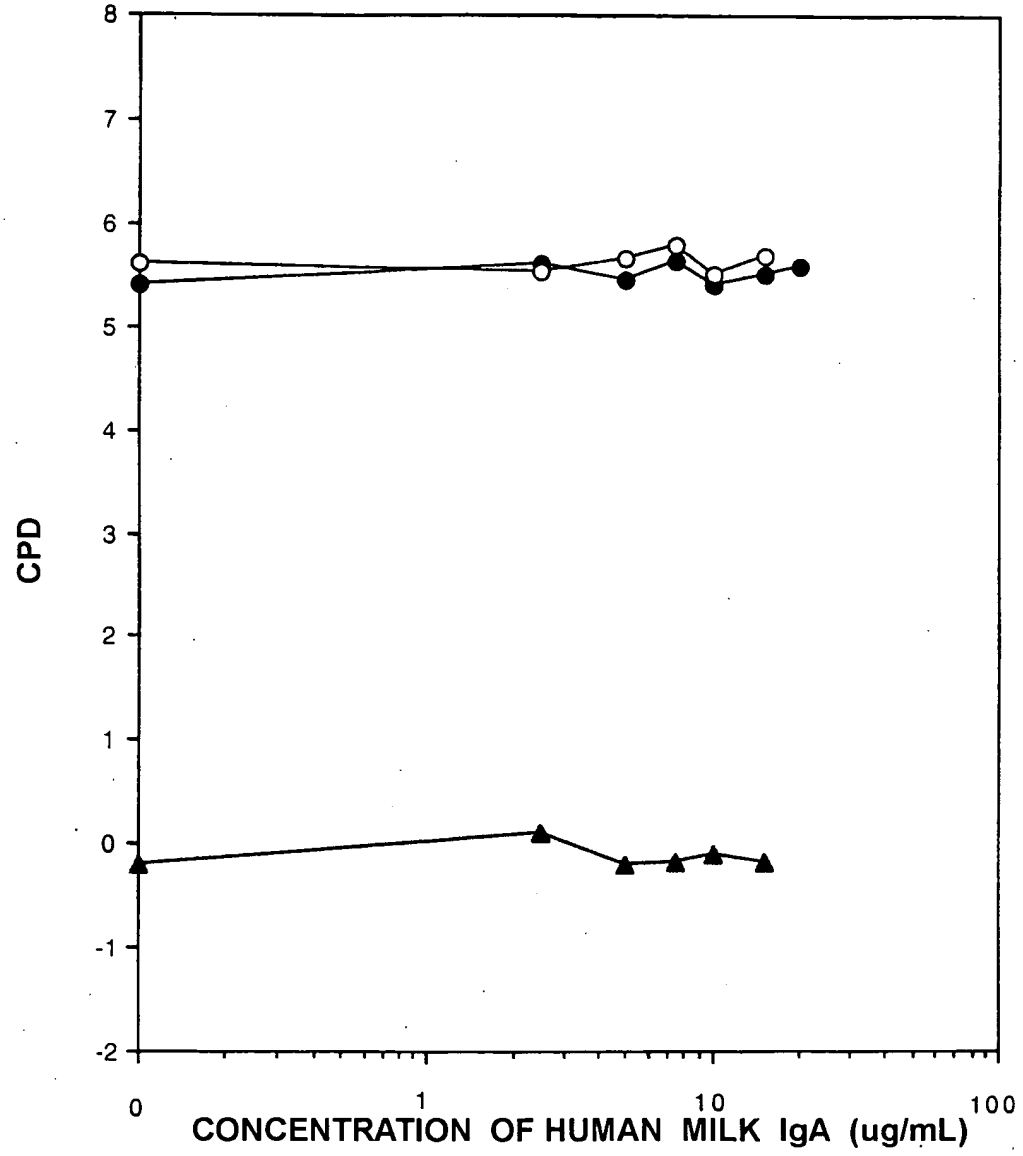
● = + E_2

■ = – E_2

▼ = Estrogenic effect

FIGURE 115

EFFECT OF HUMAN MILK SECRETORY IgA ON GH_3 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

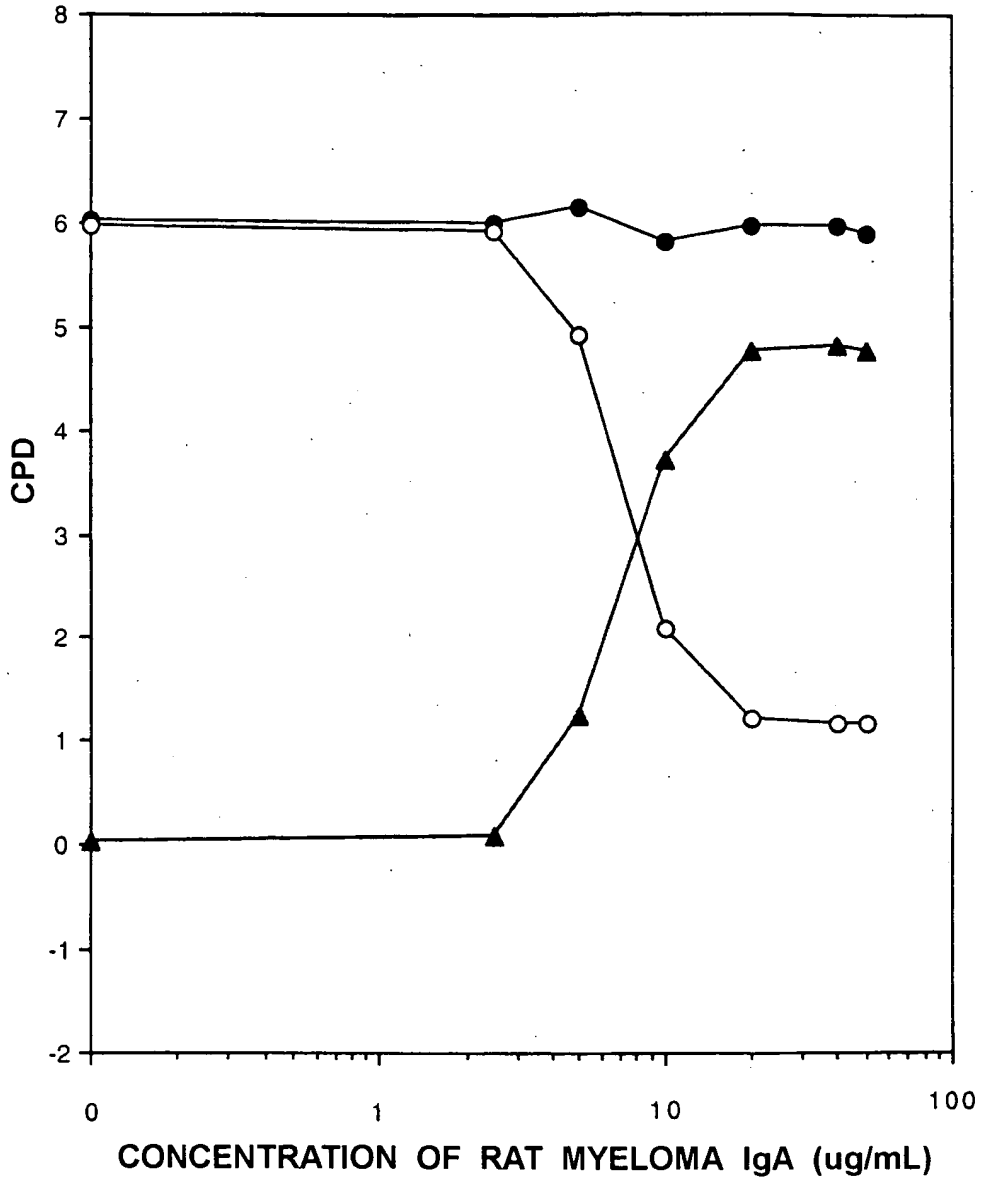
Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 116

EFFECT OF RAT MYELOMA IgA ON GH_4 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

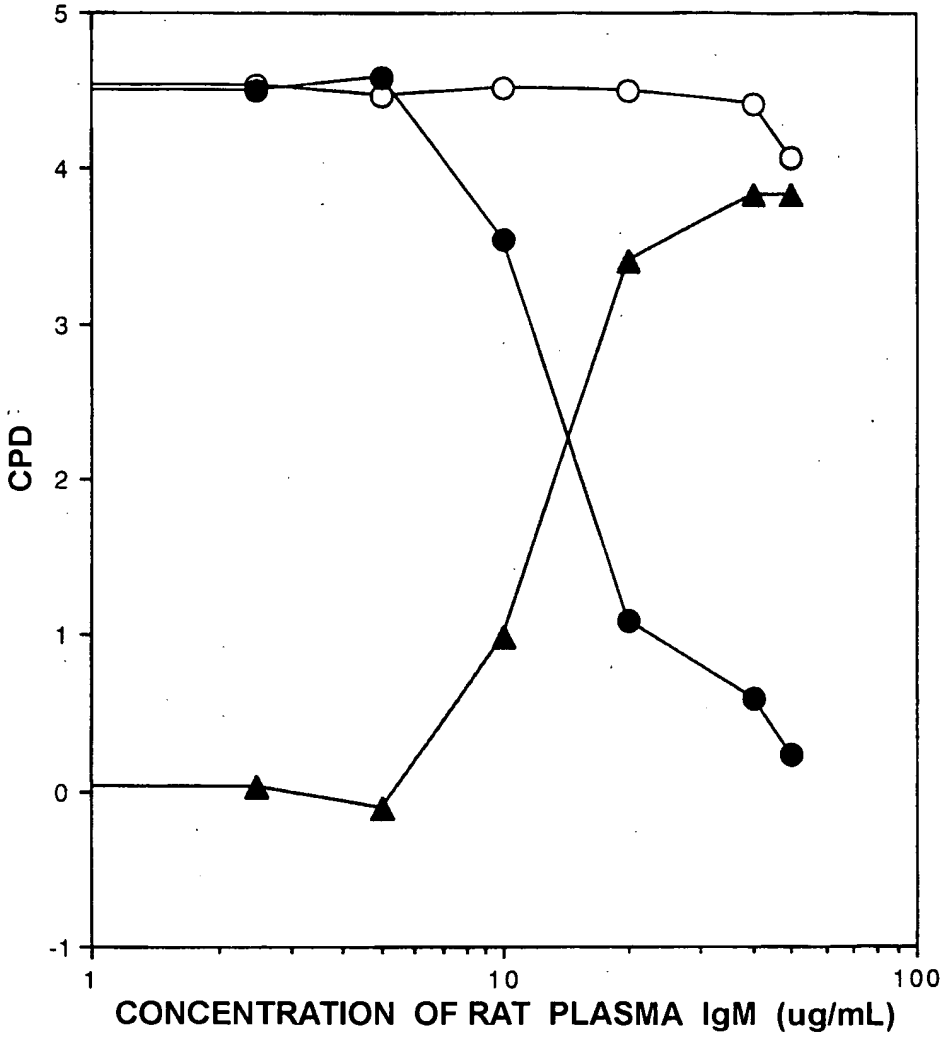
Closed circles = + E_2

Open circles = – E_2

Closed triangles = Estrogenic effect

FIGURE 117

EFFECT OF RAT PLASMA IgM ON GH_4 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

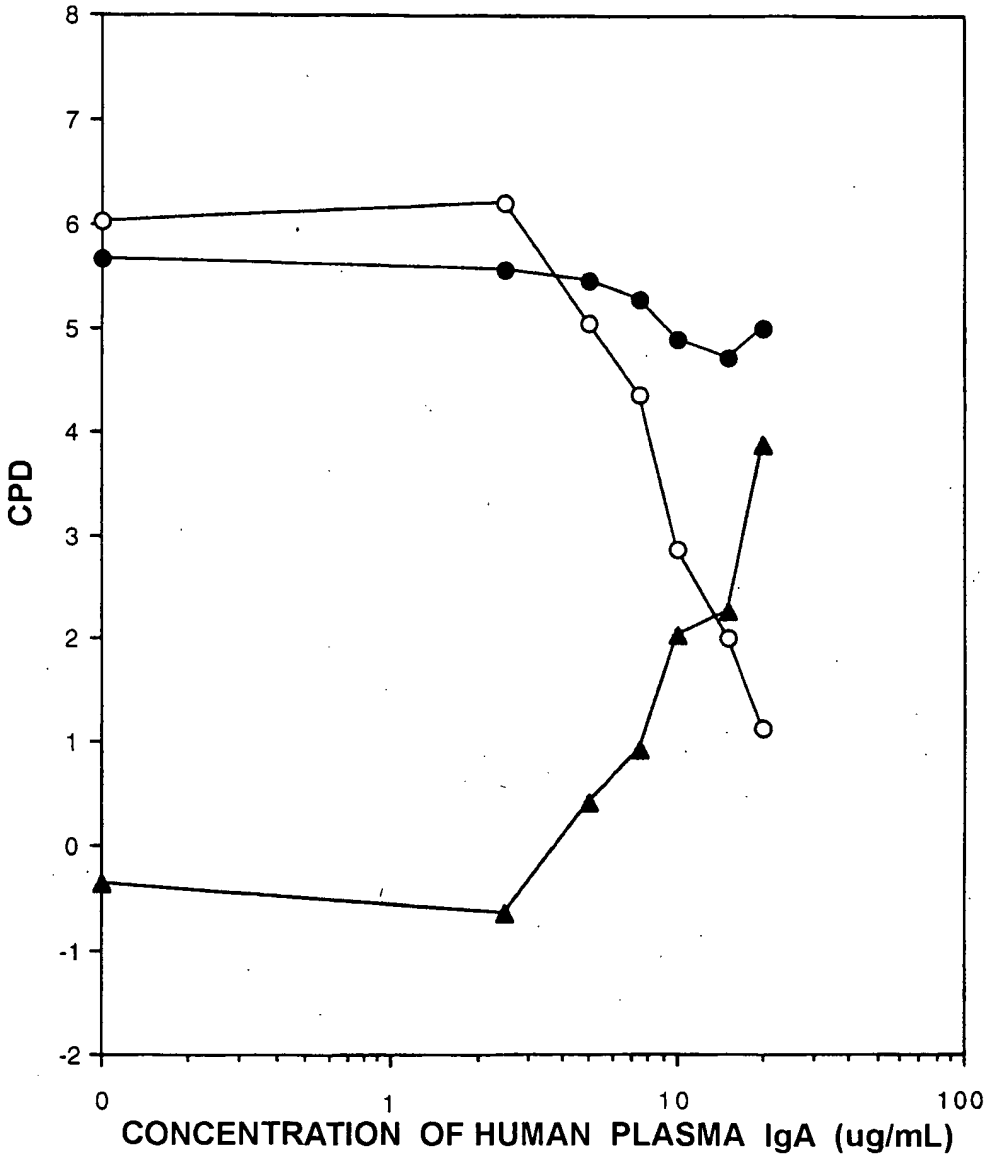
—○— = + E_2

—●— = − E_2

—▲— = Estrogenic effect

FIGURE 118

EFFECT OF HUMAN PLASMA IgA ON GH_4C_1 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

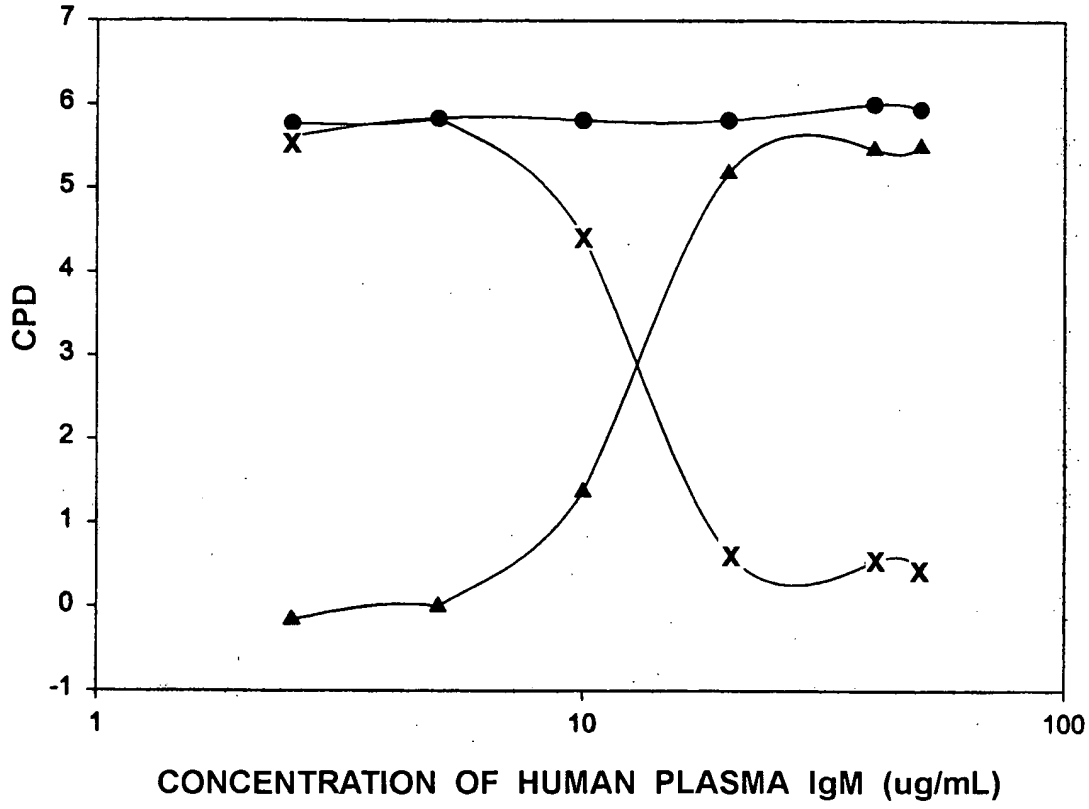
Closed circles = + E_2

Open circles = – E_2

Closed triangles = Estrogenic effect

FIGURE 119

EFFECT OF HUMAN PLASMA IgM ON GH_4C_1 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

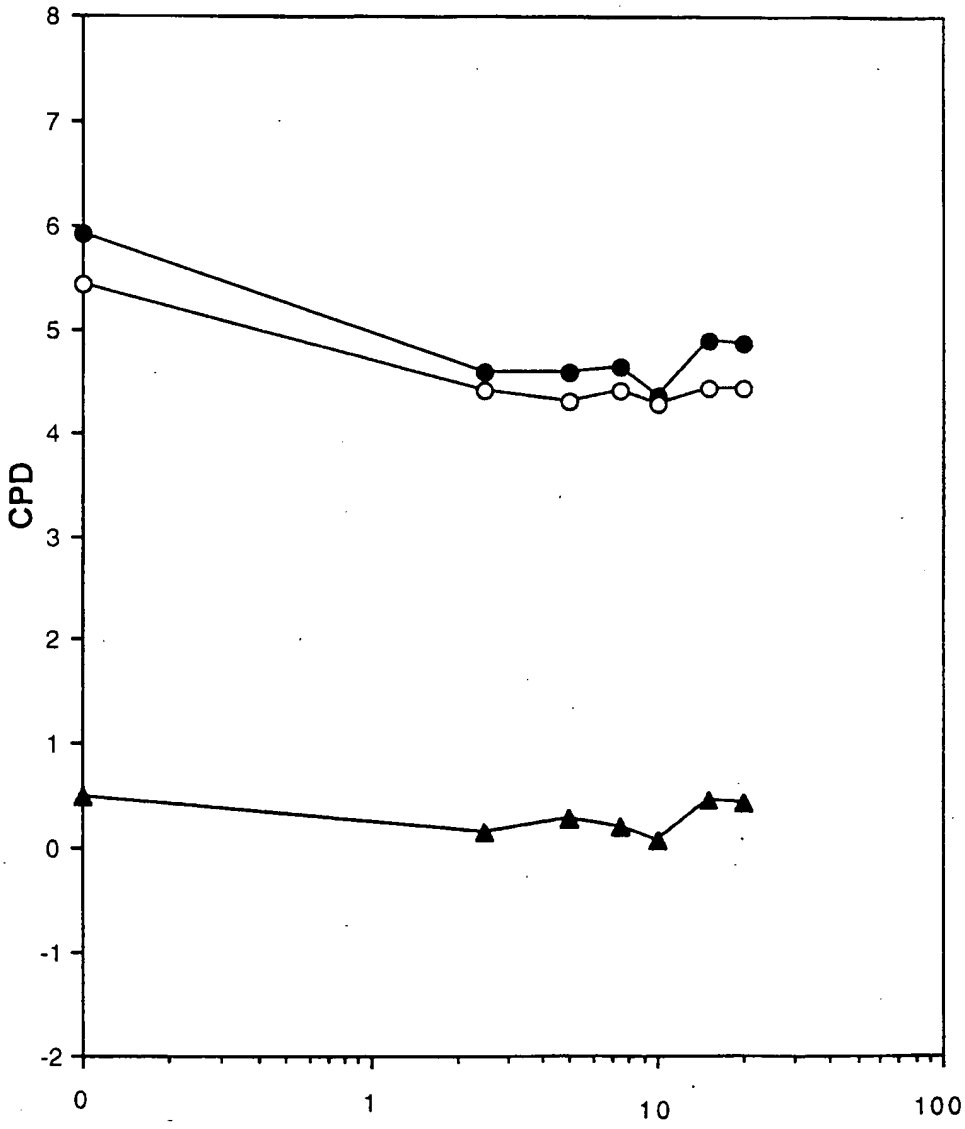
—●— = + E_2

—X— = − E_2

—▲— = Estrogenic effect

FIGURE 120

EFFECT OF HUMAN MILK SECRETORY IgA ON GH_4C_1 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

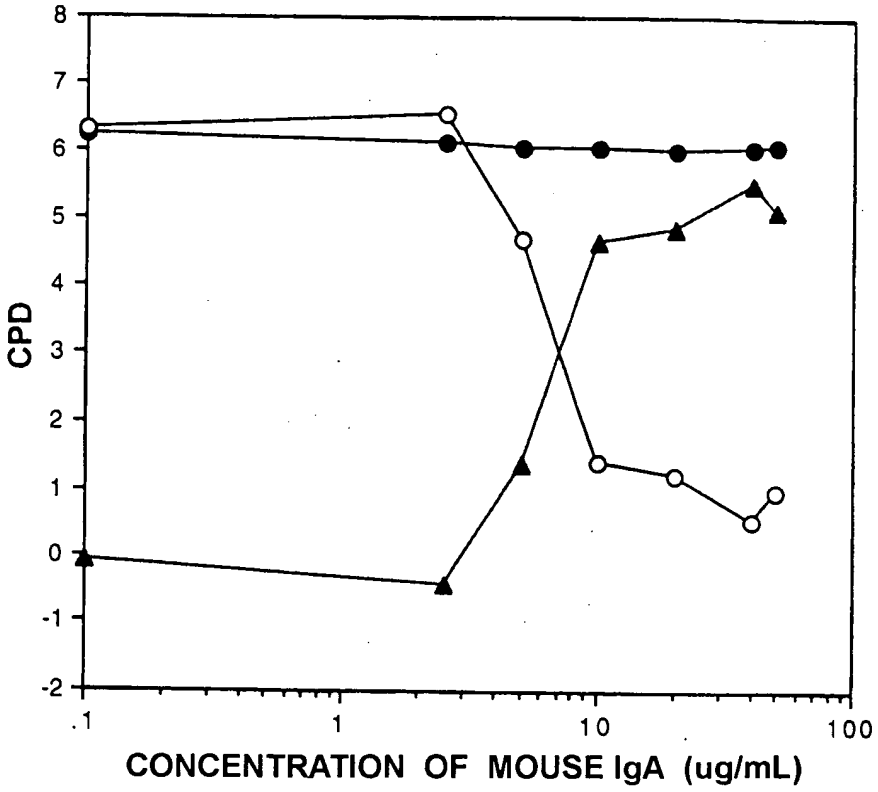
Closed circles = + E_2

Open circles = – E_2

Closed triangles = Estrogenic effect

FIGURE 121

EFFECT OF MOUSE IgA ON H301 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

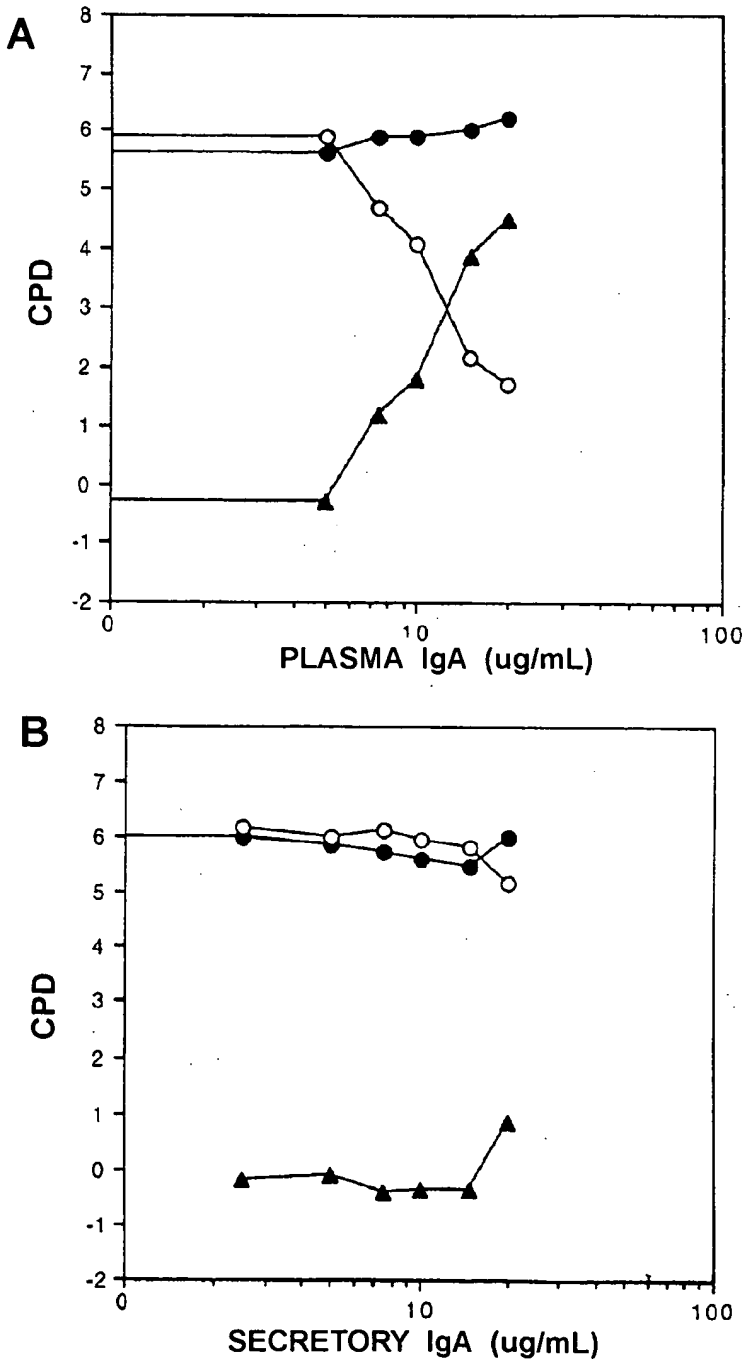
Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 122

EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON H301CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E_2
Open circles = − E_2
Closed triangles = Estrogenic effect

FIGURE 123

EFFECT OF ESTRADIOL ON H301 CELL GROWTH IN SERUM-FREE MEDIUM AND 40 ug/mL OF HUMAN IgM

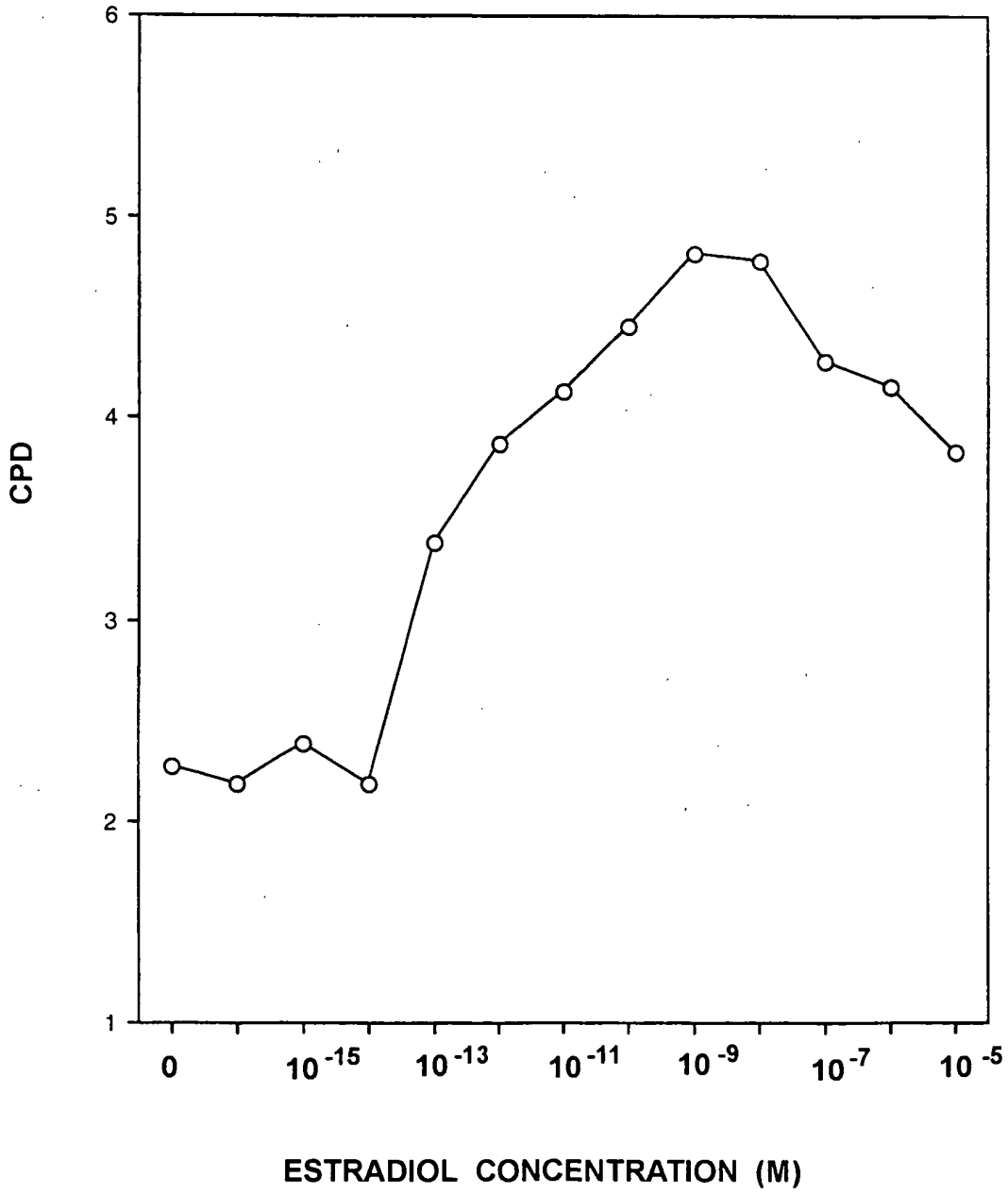
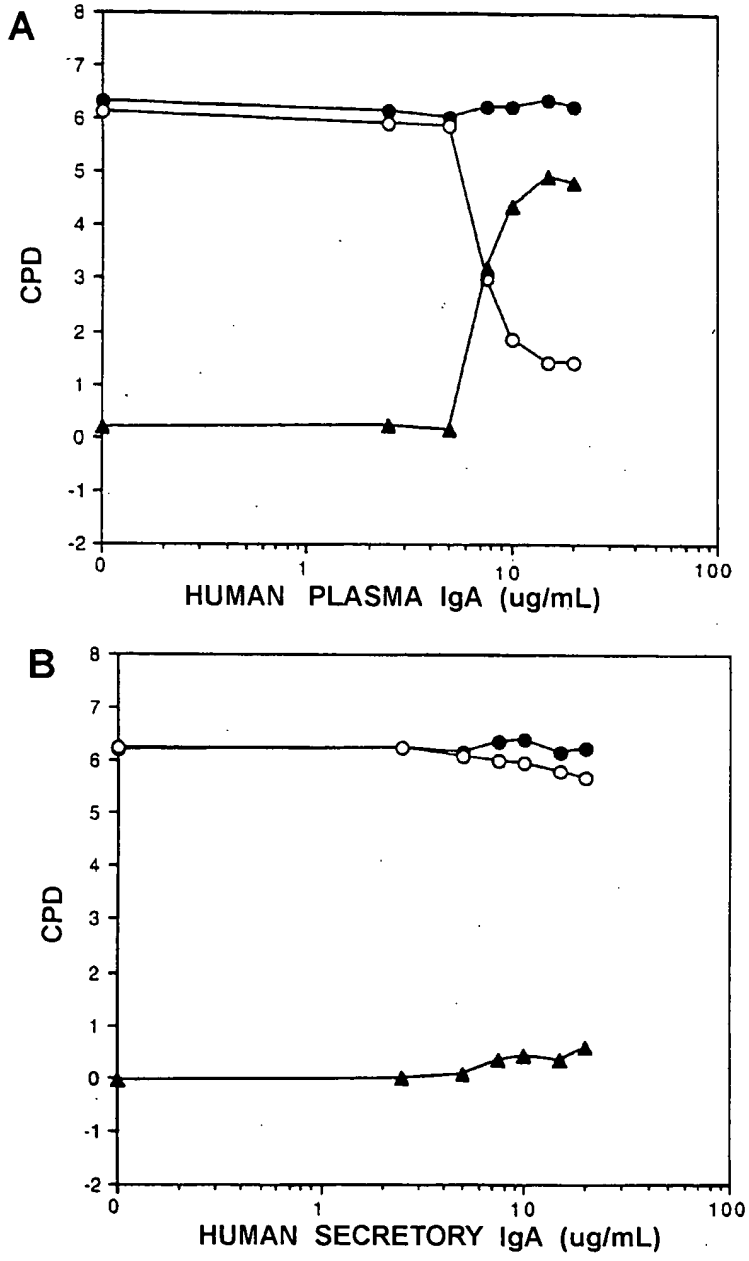


FIGURE 124

EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON MCF-7A CELL GROWTH IN SERUM-FREE MEDIUM



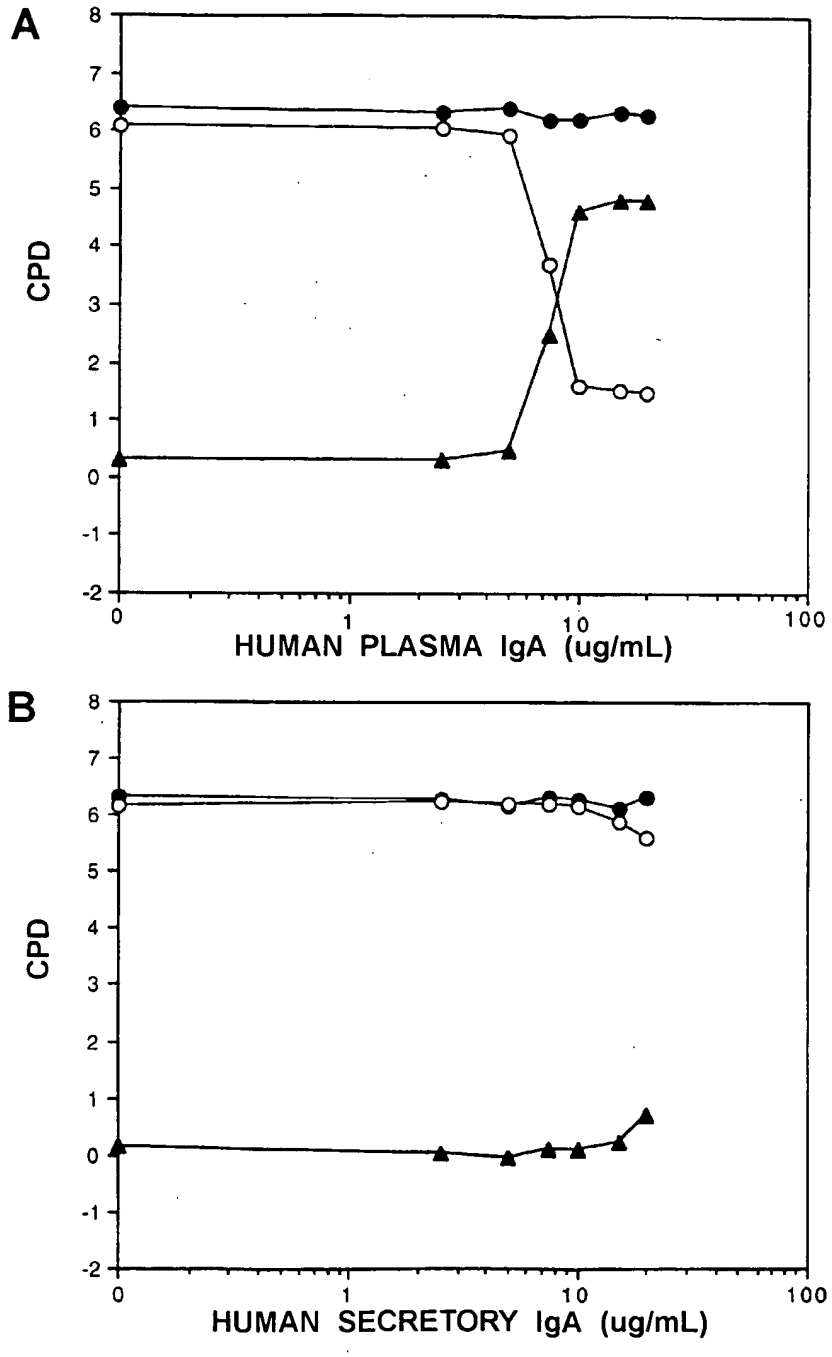
LEGEND: Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 125

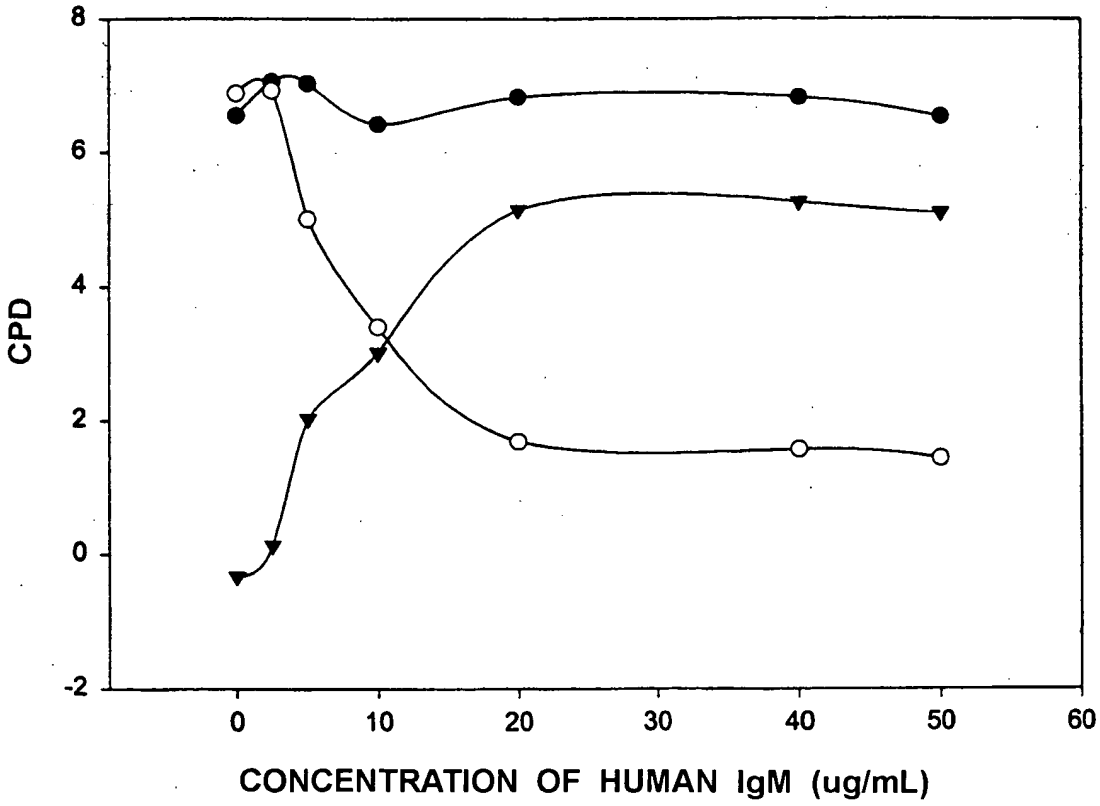
EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON MCF-7K CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E_2
Open circles = − E_2
Closed triangles = Estrogenic effect

FIGURE 126

EFFECT OF HUMAN IgM ON MCF-7A CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

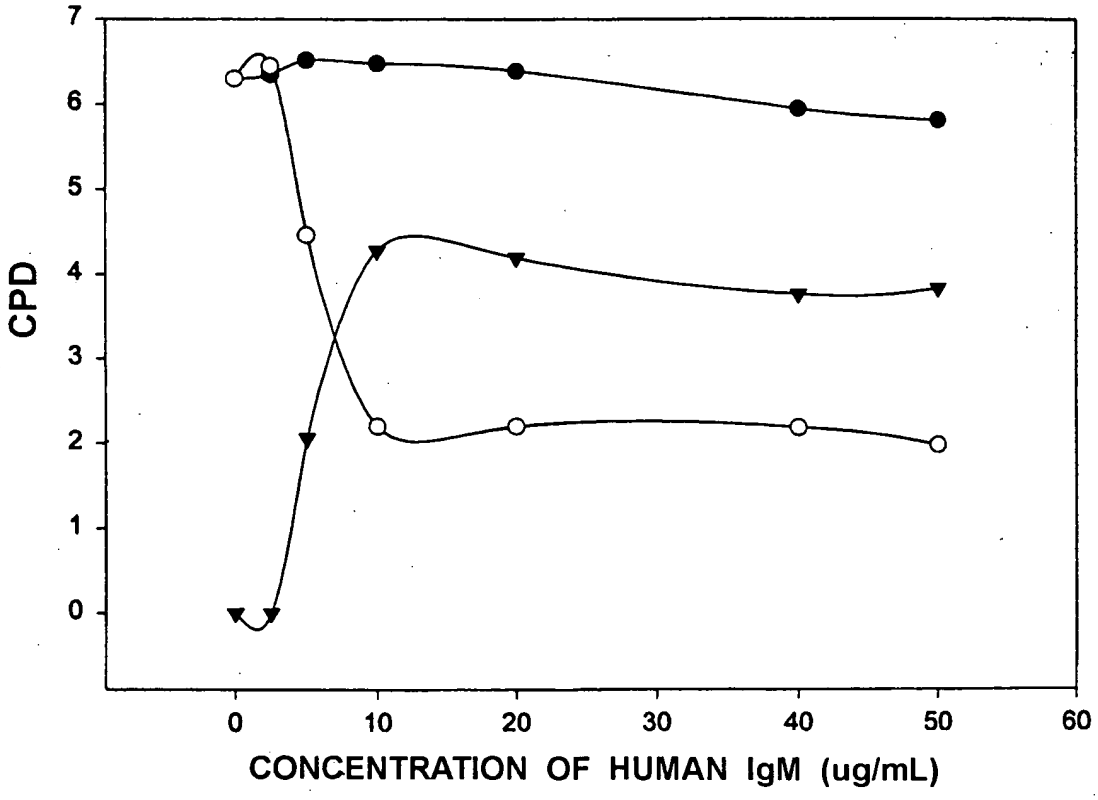
—●— = + E_2

—○— = − E_2

—▼— = Estrogenic effect

FIGURE 127

EFFECT OF HUMAN IgM ON MCF-7K CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

● = + E_2

○ = − E_2

▼ = Estrogenic effect

FIGURE 128

EFFECT OF ESTRADIOL ON MCF-7K CELL GROWTH IN SERUM-FREE MEDIUM WITH 40 ug/mL HUMAN IgM

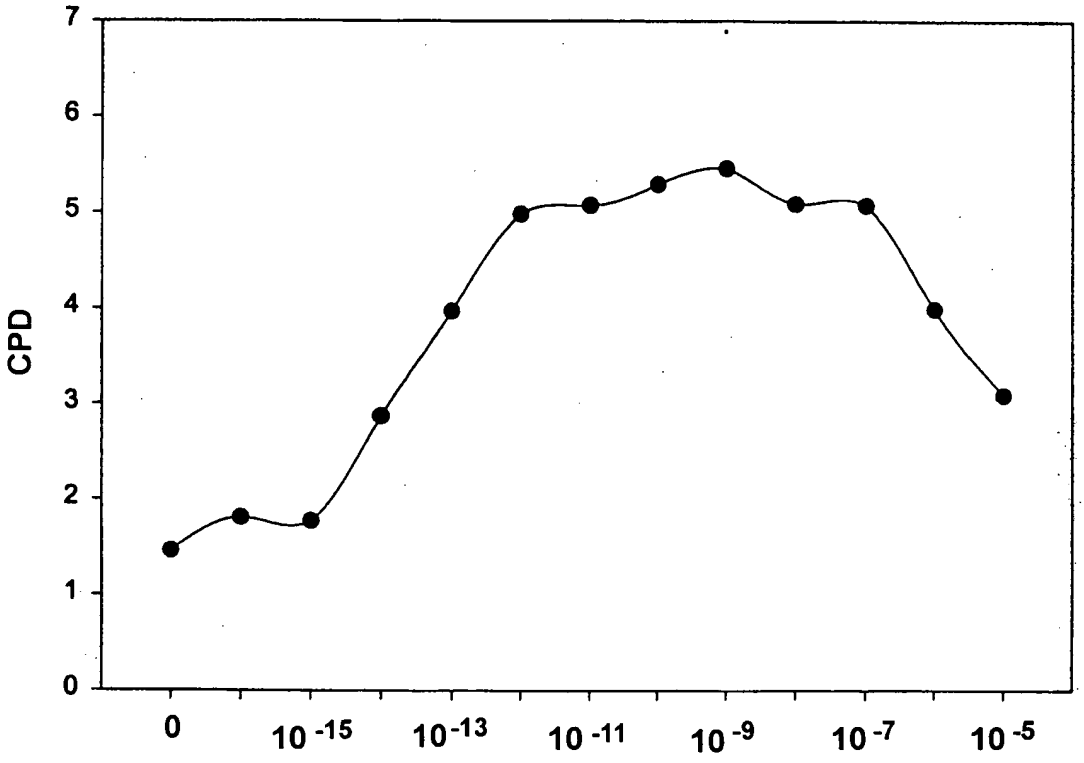
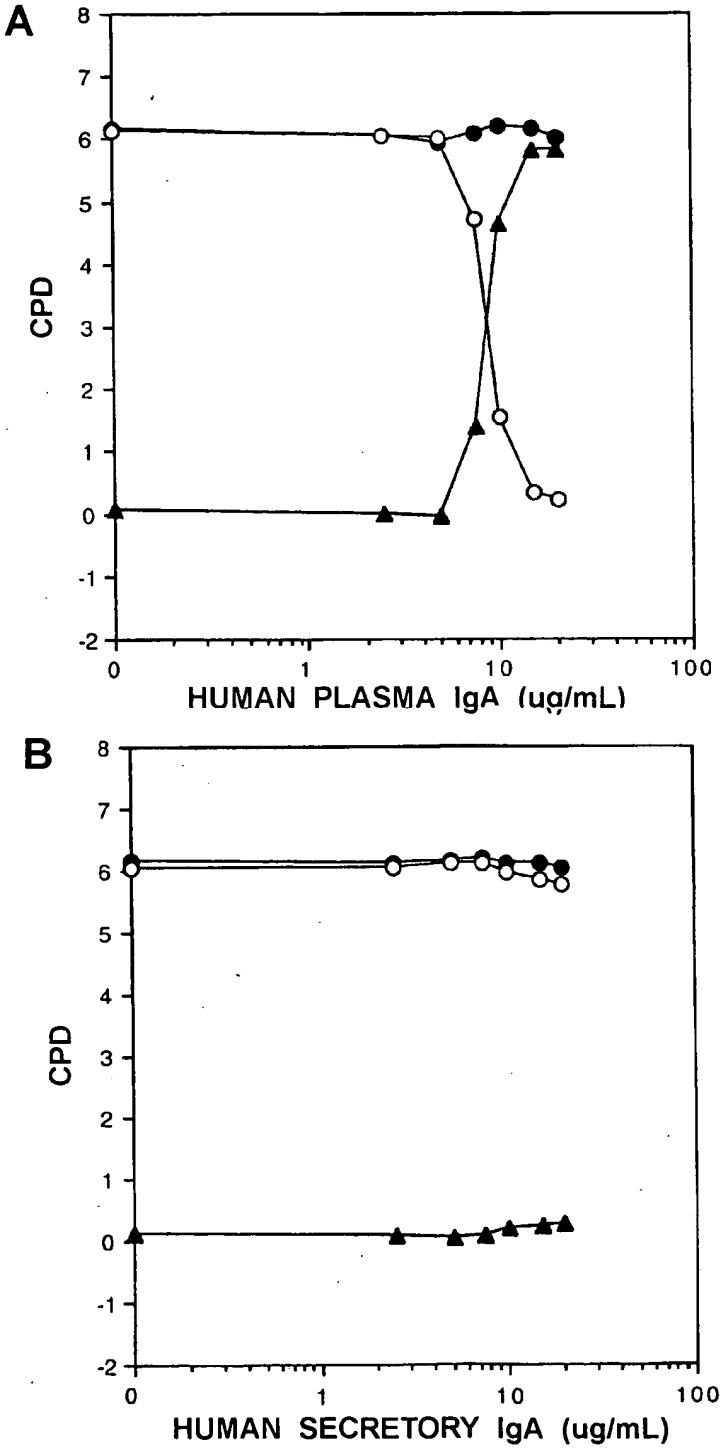


FIGURE 129

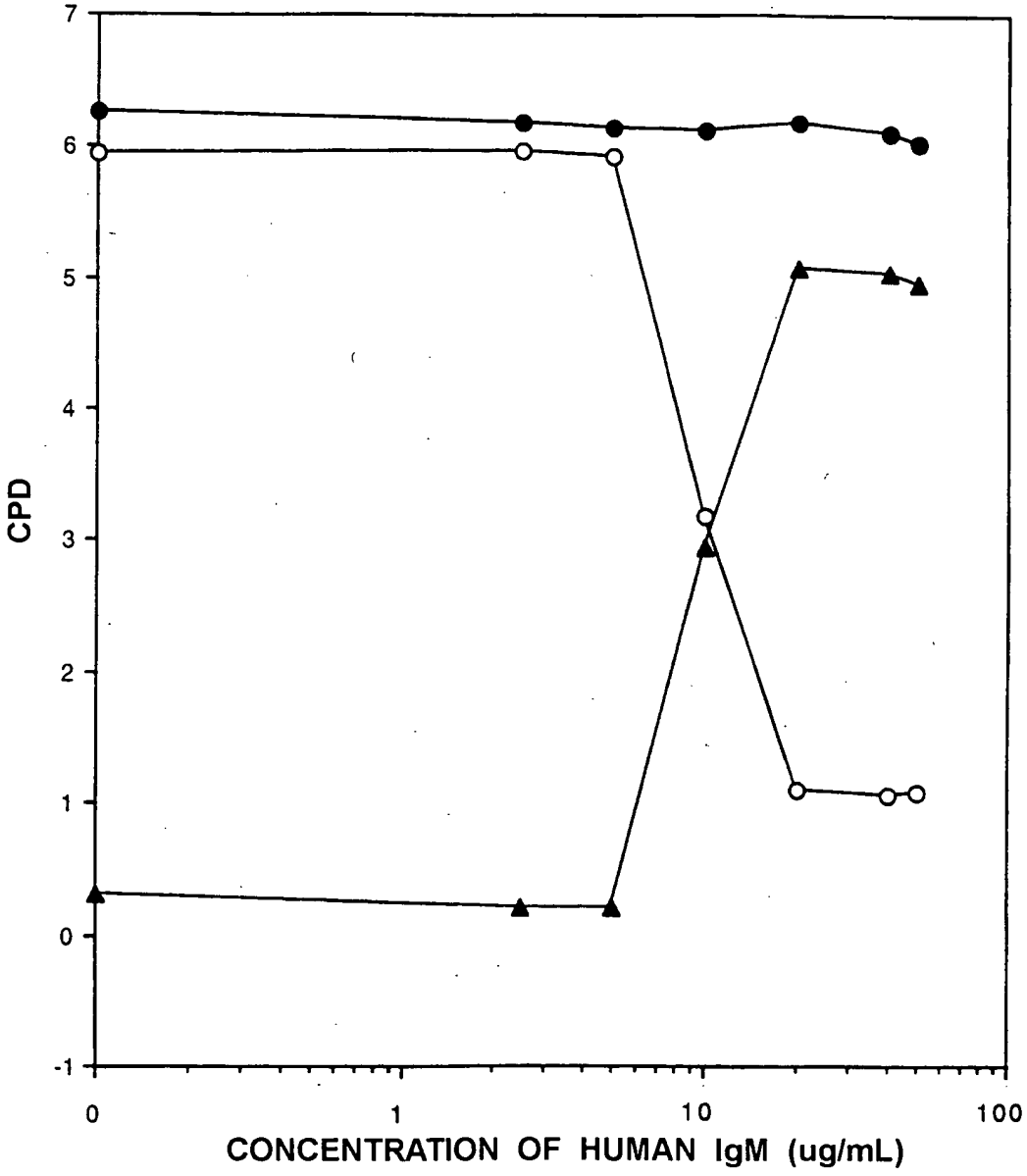
EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON T47D CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E_2
Open circles = – E_2
Closed triangles = Estrogenic effect

FIGURE 130

EFFECT OF HUMAN IgM ON T47D CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E_2

Open circles = − E_2

Closed triangles = Estrogenic effect

FIGURE 131

EFFECT OF ESTRADIOL ON T47D CELL GROWTH IN SERUM-FREE MEDIUM WITH 40 ug/mL HUMAN IgM

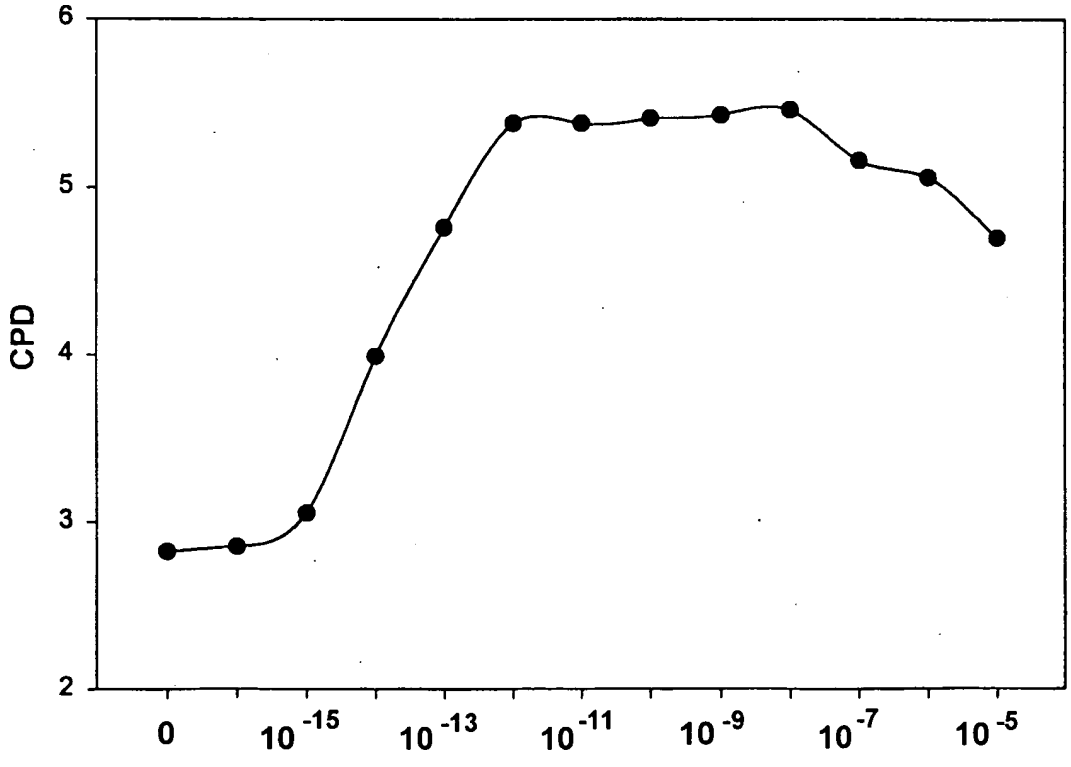
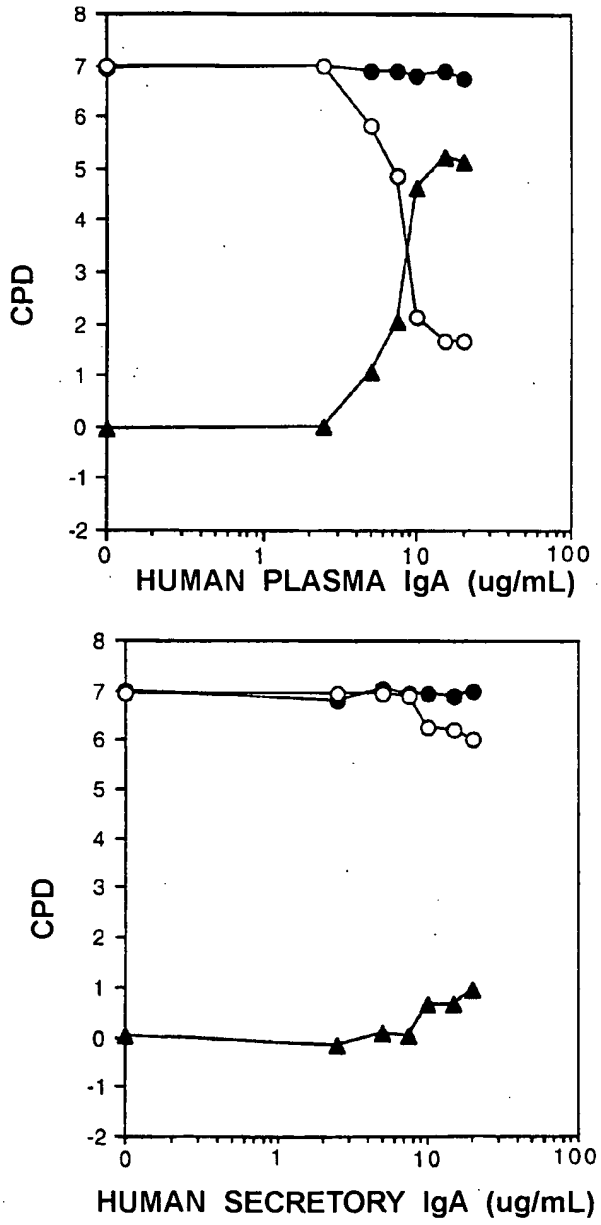


FIGURE 132

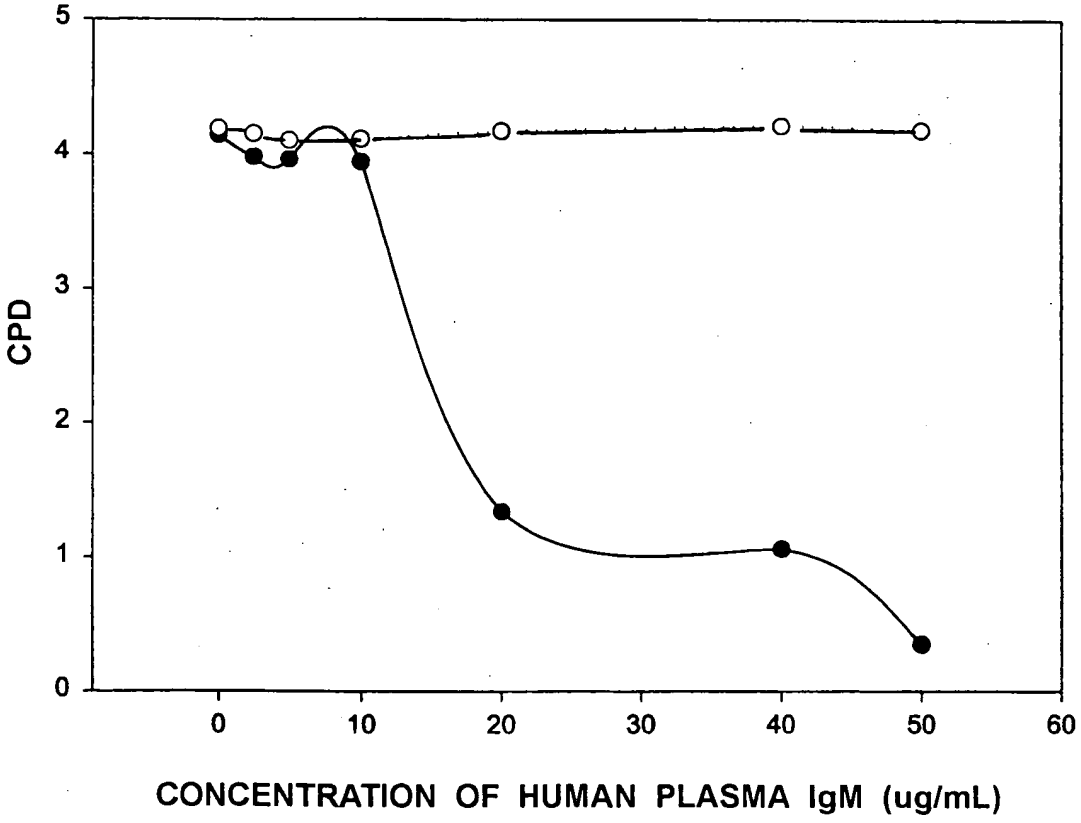
EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON ZR-75-1 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E_2
Open circles = − E_2
Closed triangles = Estrogenic effect

FIGURE 133

EFFECT OF HUMAN PLASMA IgM ON ZR-75-1 CELL GROWTH IN SERUM-FREE MEDIUM



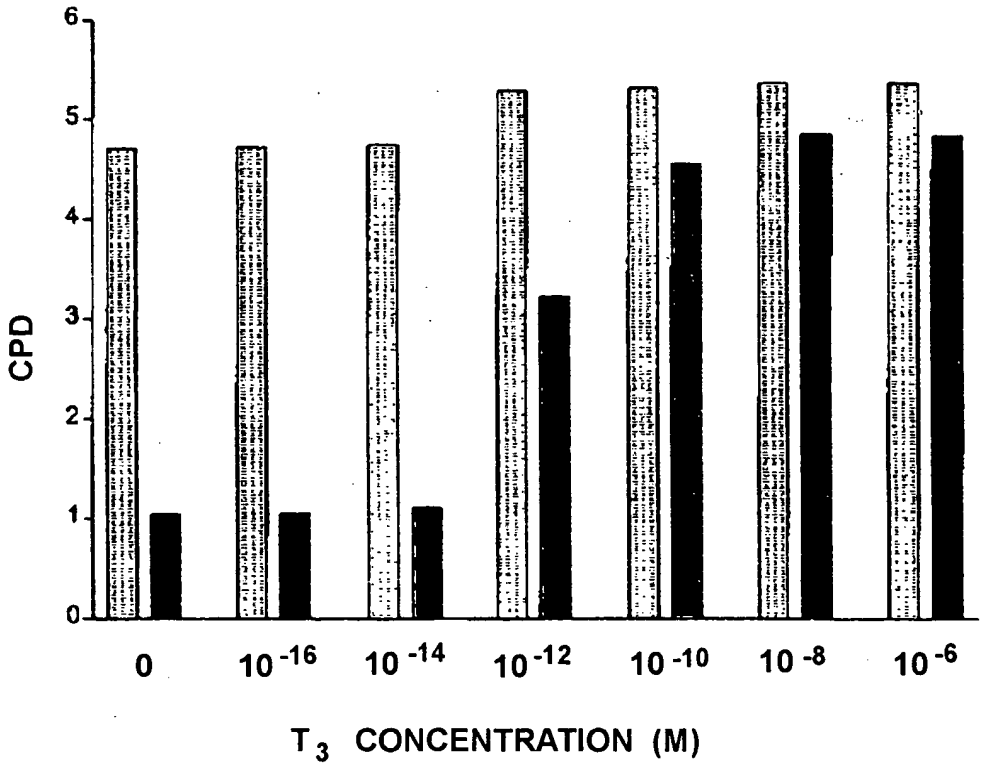
LEGEND:

—●— = – E_2

–○– = + E_2

FIGURE 134

EFFECT OF HUMAN IgM ON HT-29 CELL GROWTH IN THE PRESENCE OF INCREASING CONCENTRATIONS OF T_3



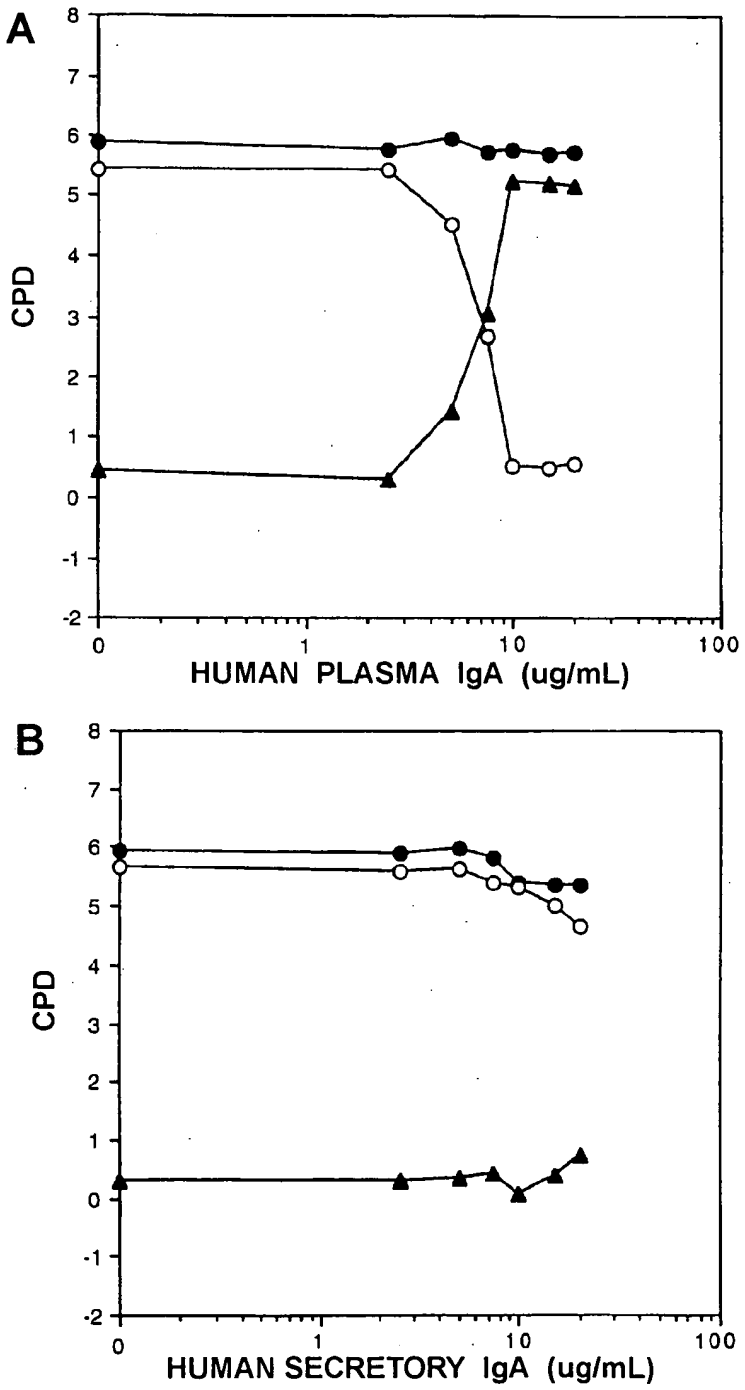
LEGEND:

= T_3 Titration

= T_3 Titration + 40 ug/mL IgM

FIGURE 135

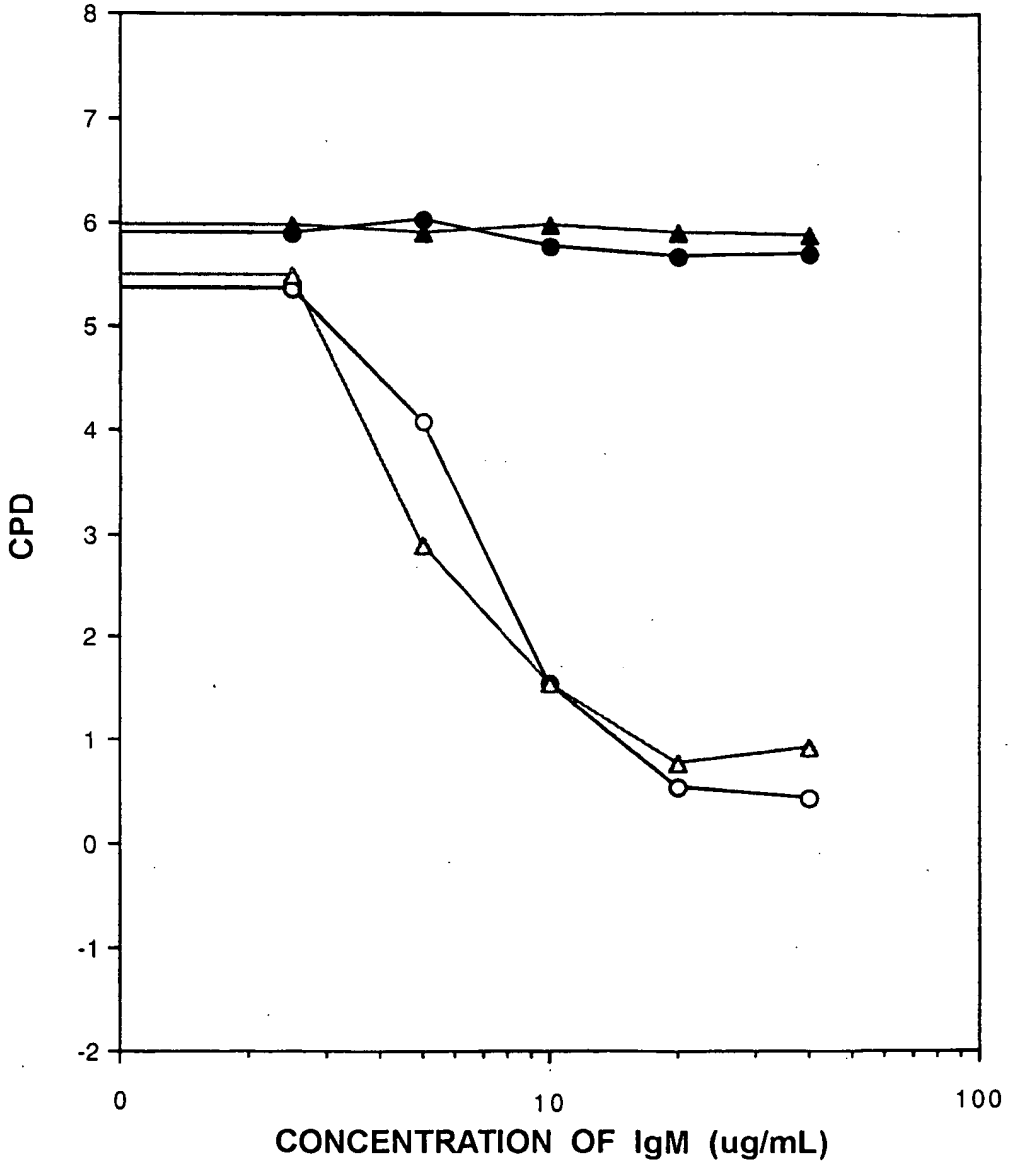
EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY IgA (B) ON LNCaP CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: Closed circles = + E_2
Open circles = − E_2
Closed triangles = Estrogenic effect

FIGURE 136

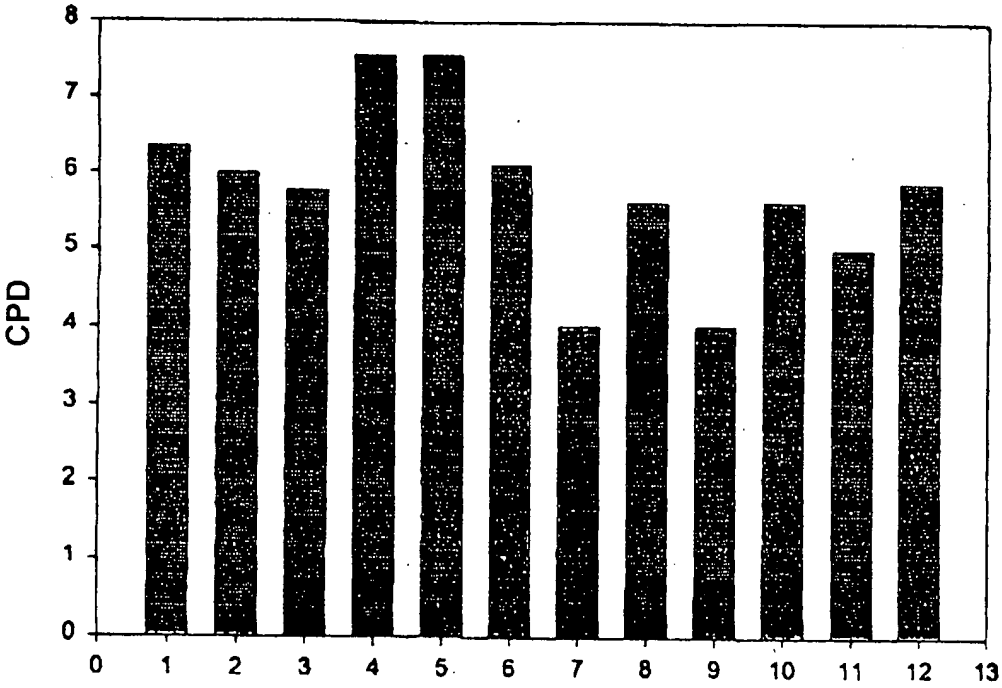
EFFECTS OF HUMAN PLASMA IgM VS IgM DERIVED FROM MYELOMA CELLS ON LNCaP CELL GROWTH IN SERUM-FREE MEDIUM WITH AND WITHOUT DHT



LEGEND:
—●— = DHT + Myeloma IgM
—○— = Myeloma IgM only
—▲— = DHT + Plasma IgM
—△— = Plasma IgM only

FIGURE 137

ESTROGENIC EFFECT OF 50 ug/mL OF VARIOUS IgM'S ON SEVERAL DIFFERENT CELL LINES

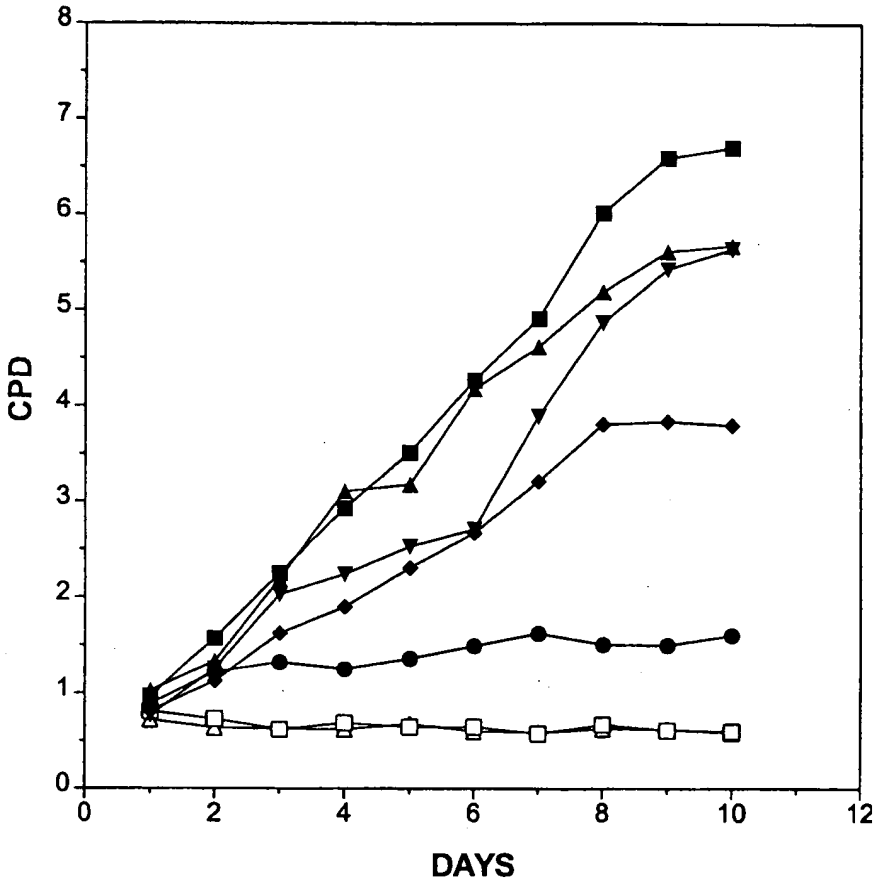


LEGEND:

1. Human IgM on MTW9/PL2 Cells = 6.36 cpd
2. Mouse IgM on MTW9/PL2 Cells = 6.00 cpd
3. Rat IgM on MTW9/PL2 Cells = 5.77 cpd
4. Human IgM on H301 Cells = 7.57 cpd
5. Mouse IgM on H301 Cells = 7.56 cpd
6. Rat IgM on H301 Cells = 6.11 cpd
7. Human IgM on GH1 Cells = 4.12 cpd
8. Rat IgM on GH1 Cells = 5.83 cpd
9. Human IgM on GH3 Cells = 4.09 cpd
10. Human IgM on GH4 Cells = 5.41 cpd
11. Human IgM on MCF-7A Cells = 5.01 cpd
12. Human IgM on MCF-7K Cells = 5.89 cpd

FIGURE 138

EFFECT OF TAMOXIFEN ON T47D CELL GROWTH IN DDM-2MF DEFINED MEDIUM

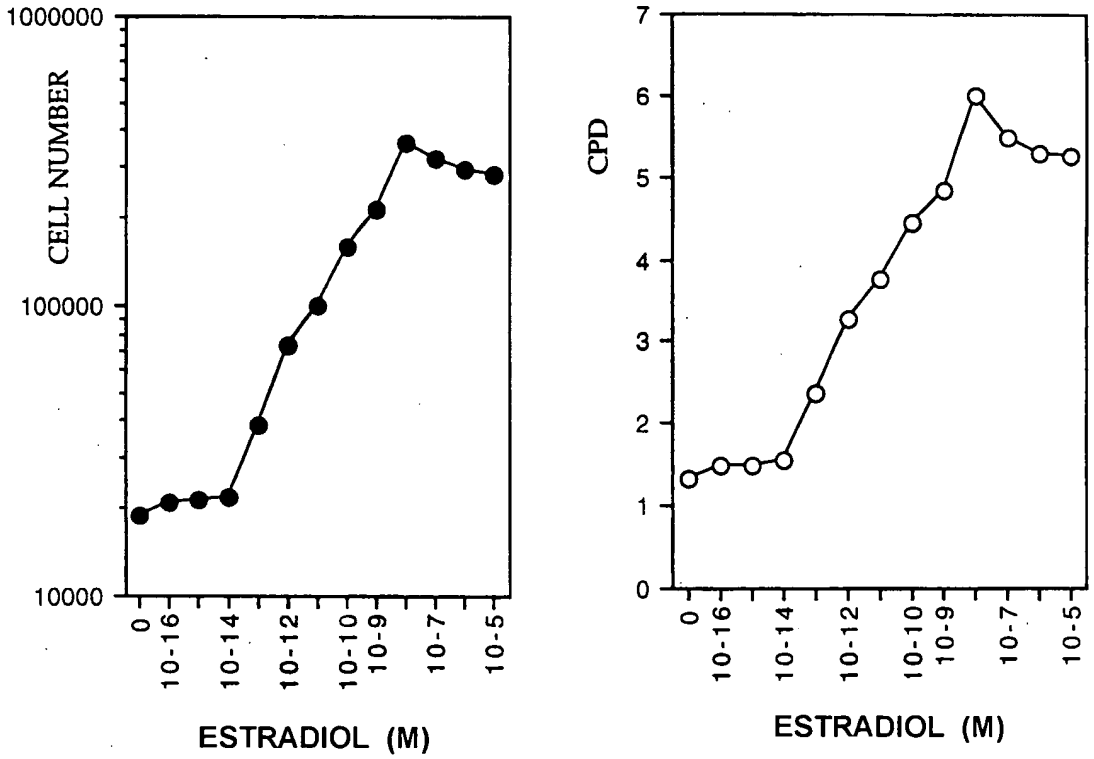


LEGEND:

- SFM + E_2
- SFM - E_2
- SFM + 10^{-9} M TAM
- SFM + 10^{-8} M TAM
- SFM + 10^{-7} M TAM
- SFM + 10^{-6} M TAM
- SFM + 10^{-5} M TAM

FIGURE 139

EFFECT OF INCREASING ESTRADIOL CONCENTRATIONS ON T47D CELL GROWTH IN SERUM-FREE AND PHENOL- RED FREE MEDIUM WITH 10^{-7} TAMOXIFEN

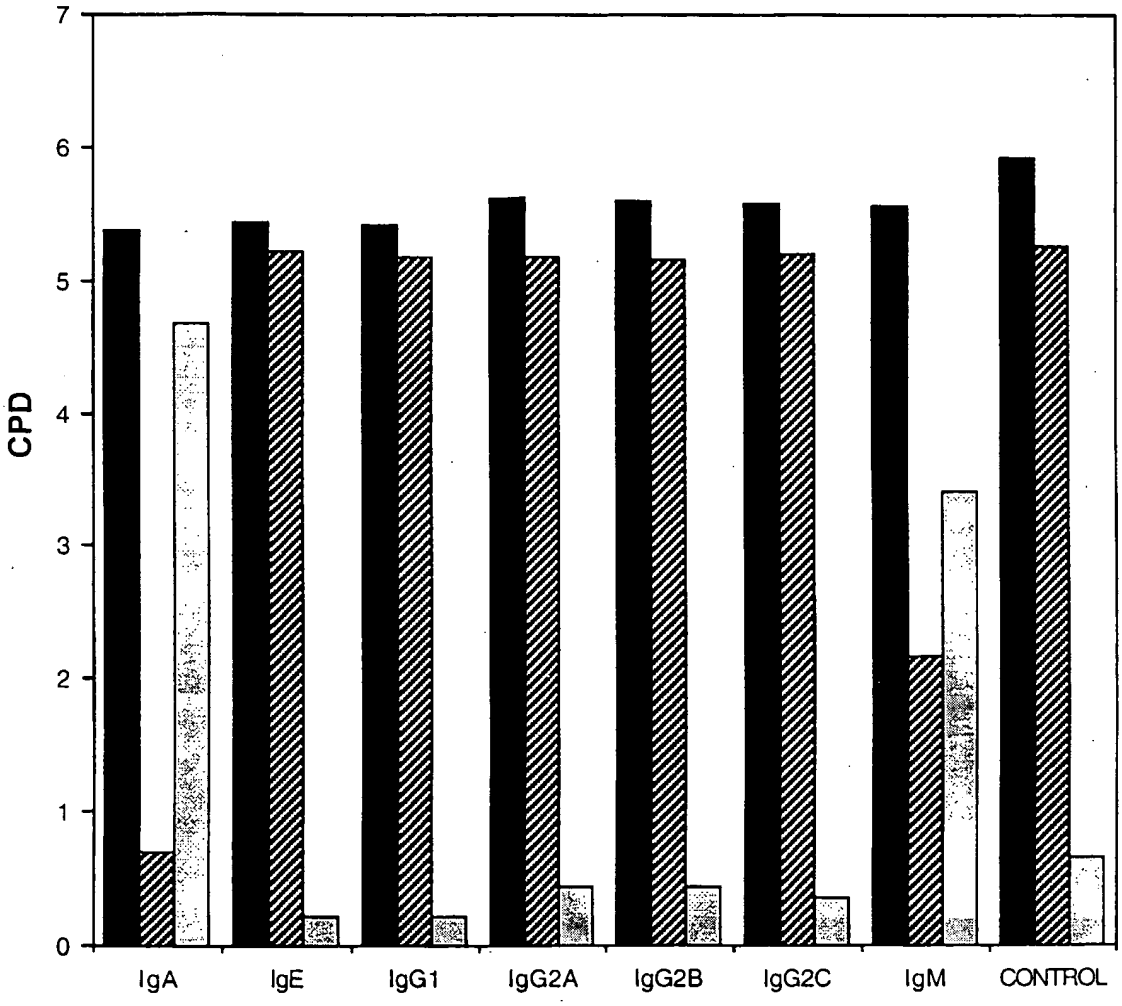


NOTE:

DATA ARE EXPRESSED AS BOTH CELL NUMBER AND CPD

FIGURE 140

EFFECT OF RAT IMMUNOGLOBULINS ON MTW9/PL2 CELL GROWTH IN SERUM-FREE MEDIUM



CONCENTRATION OF RAT IMMUNOGLOBULINS (15 ug/mL)

LEGEND:

■ = + E_2

▨ = − E_2

▩ = Estrogenic effect

CONTROL IS SERUM-FREE MEDIUM ALONE $\pm E_2$

FIGURE 141

ESTROGENIC EFFECT GENERATED BY IMMUNOGLOBULINS WITH T47D CELLS IN SERUM-FREE MEDIUM

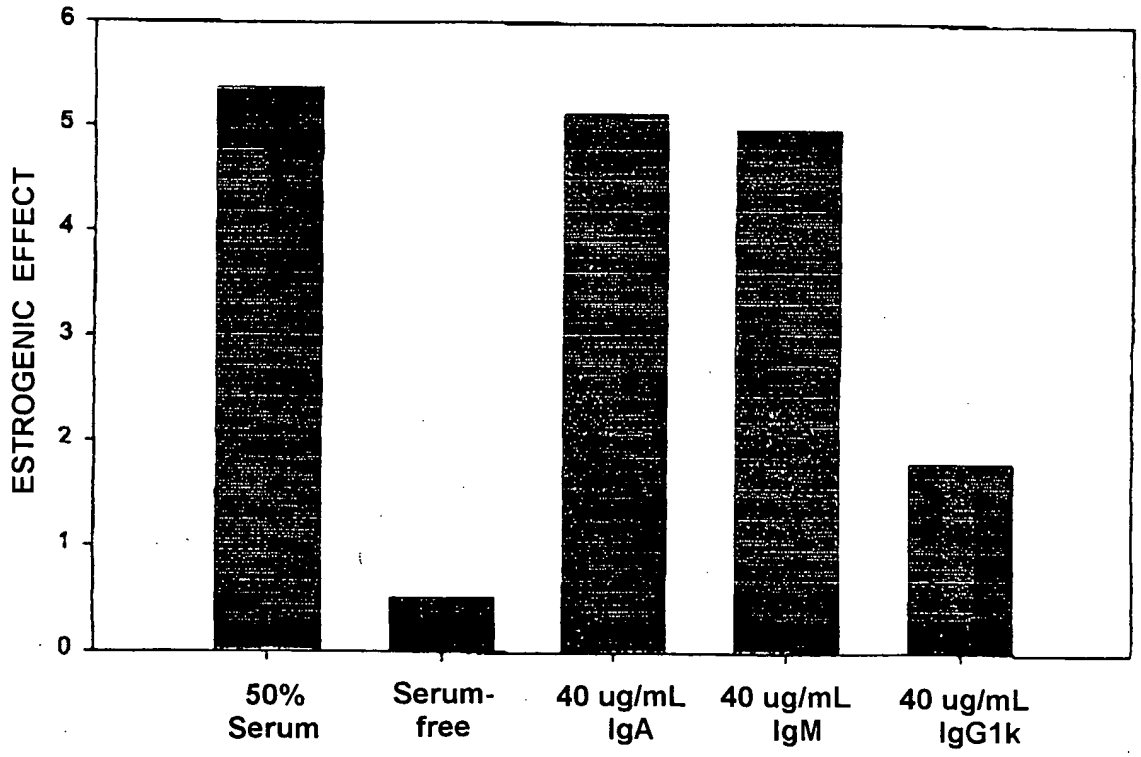
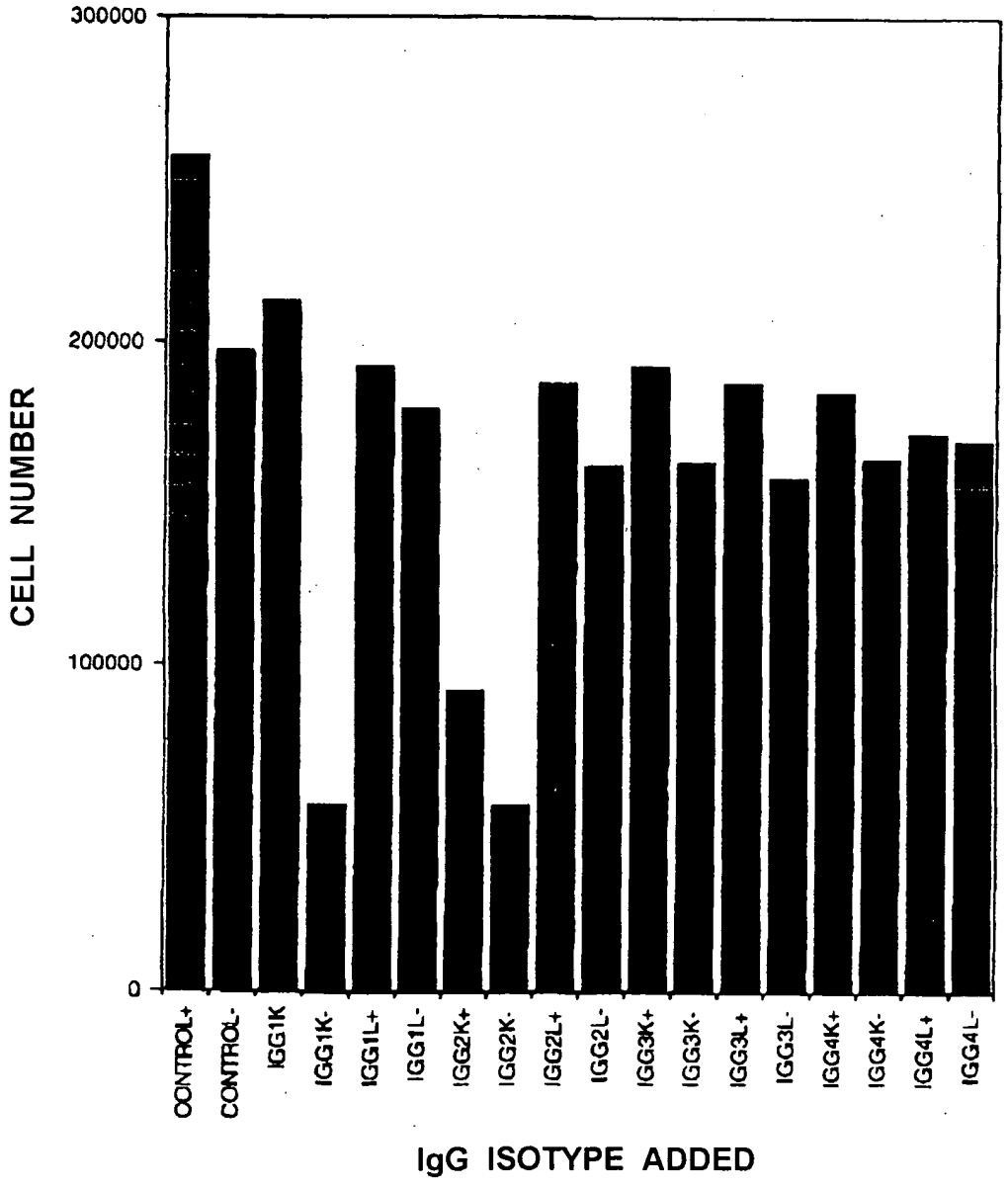


FIGURE 142

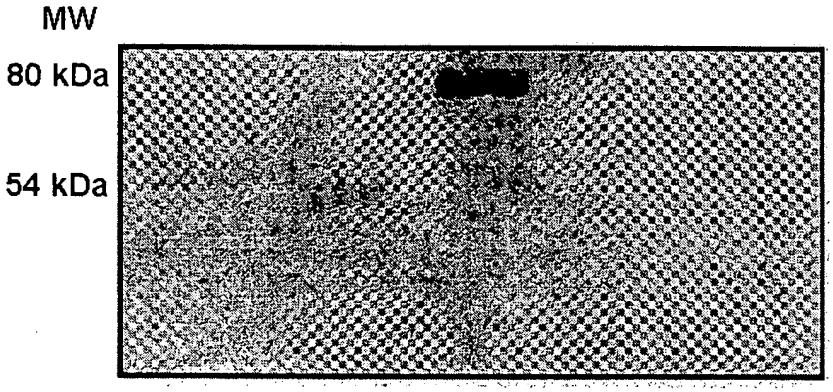
EFFECT OF IgG ISOTYPES (40 ug/mL) ON LNCaP CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND: + = DHT Added
– = No DHT Added

FIGURE 143

DETECTION OF SECRETORY COMPONENT IN SECRETORY IgA WITH ANTI-SC ANTIBODY



20 ug/mL APPLIED IN ALL LINES

IgA = Human Plasma

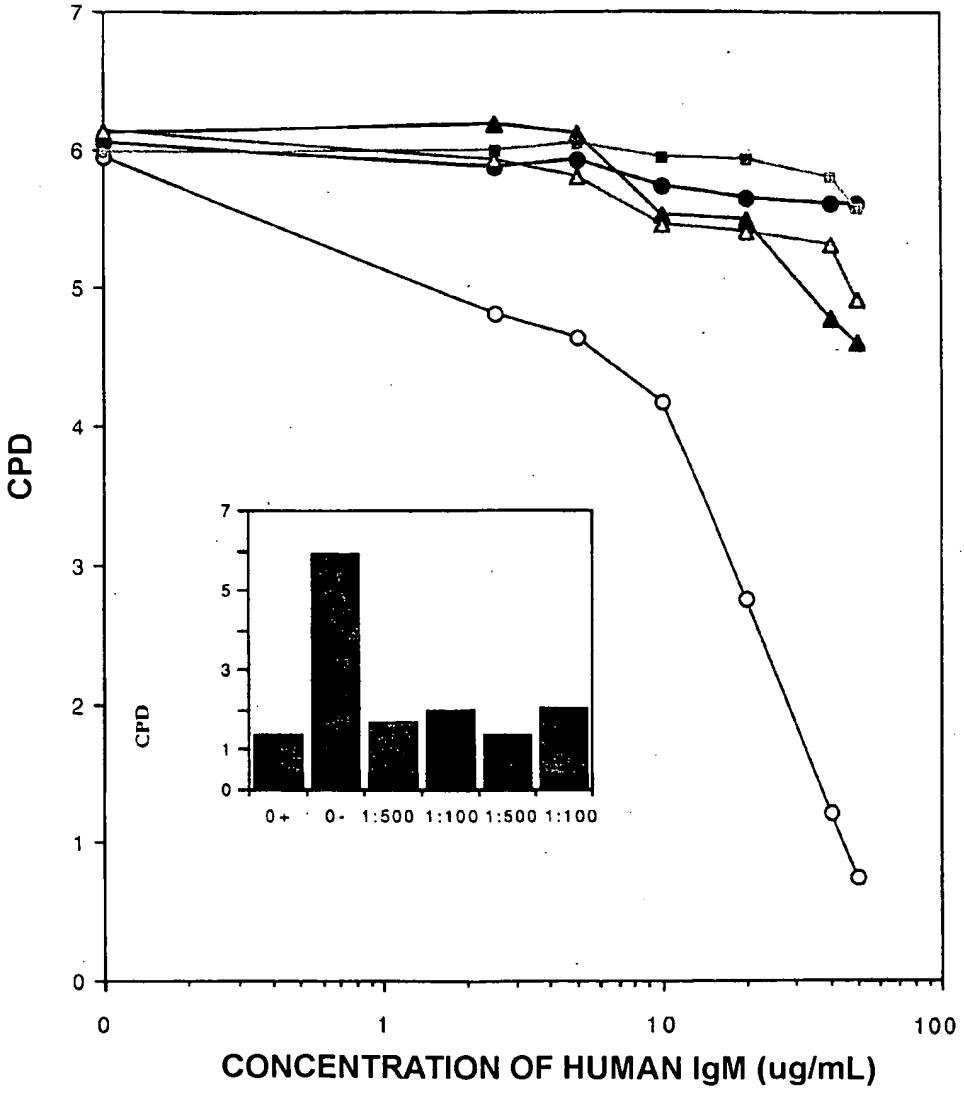
IgG = Human Plasma

Secretory IgA = IgA from Milk

Albumin = Human

FIGURE 144

HUMAN IgM TITRATION ON T47D CELLS GROWN IN SERUM-FREE MEDIUM WITH DIFFERENT DILUTIONS OF ANTI-SC ANTIBODY

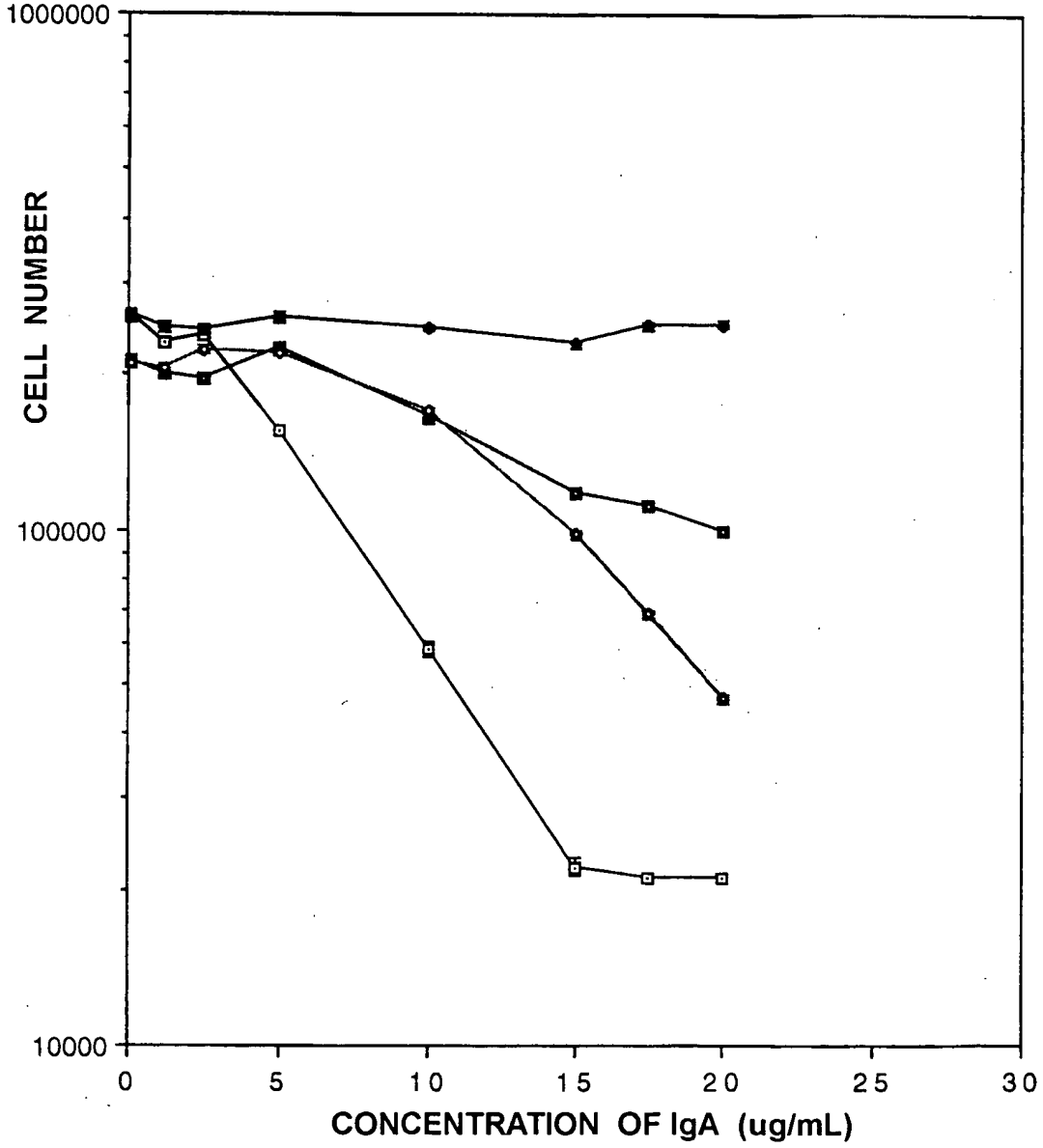


LEGEND: —●— = + E_2
—○— = − E_2
—▲— = 1:5000 Dilution of Anti-SC Antibody
—△— = 1:1000 Dilution of Anti-SC Antibody
—■— = 1:500 Dilution of Anti-SC Antibody

INSERT: EFFECT OF RABBIT SERUM ON T47D CELLS INCUBATED WITH 40 ug/mL HUMAN IgM

FIGURE 145

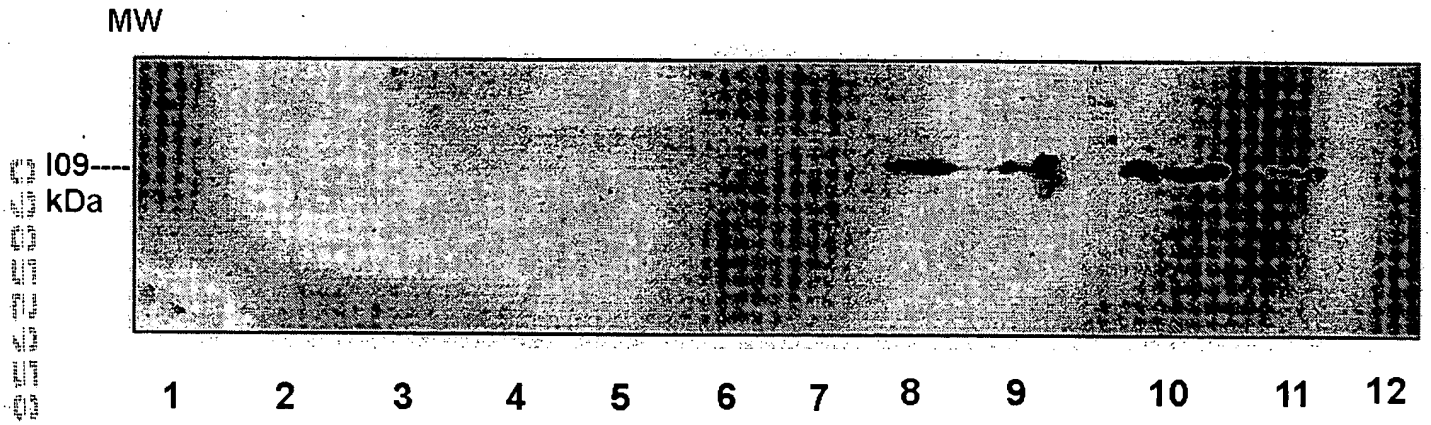
EFFECT OF IgA ON LNCaP GROWTH IN THE PRESENCE OF ANTI-SECRETORY COMPONENT ANTIBODY AT DIFFERENT DILUTIONS



LEGEND:
= Control
= 1:100 Dilution of Anti-SC Antibody
= 1:500 Dilution of Anti-SC Antibody
= 1:1000 Dilution of Anti-SC Antibody

FIGURE 146

WESTERN BLOT: ANTI-SECRETORY COMPONENT

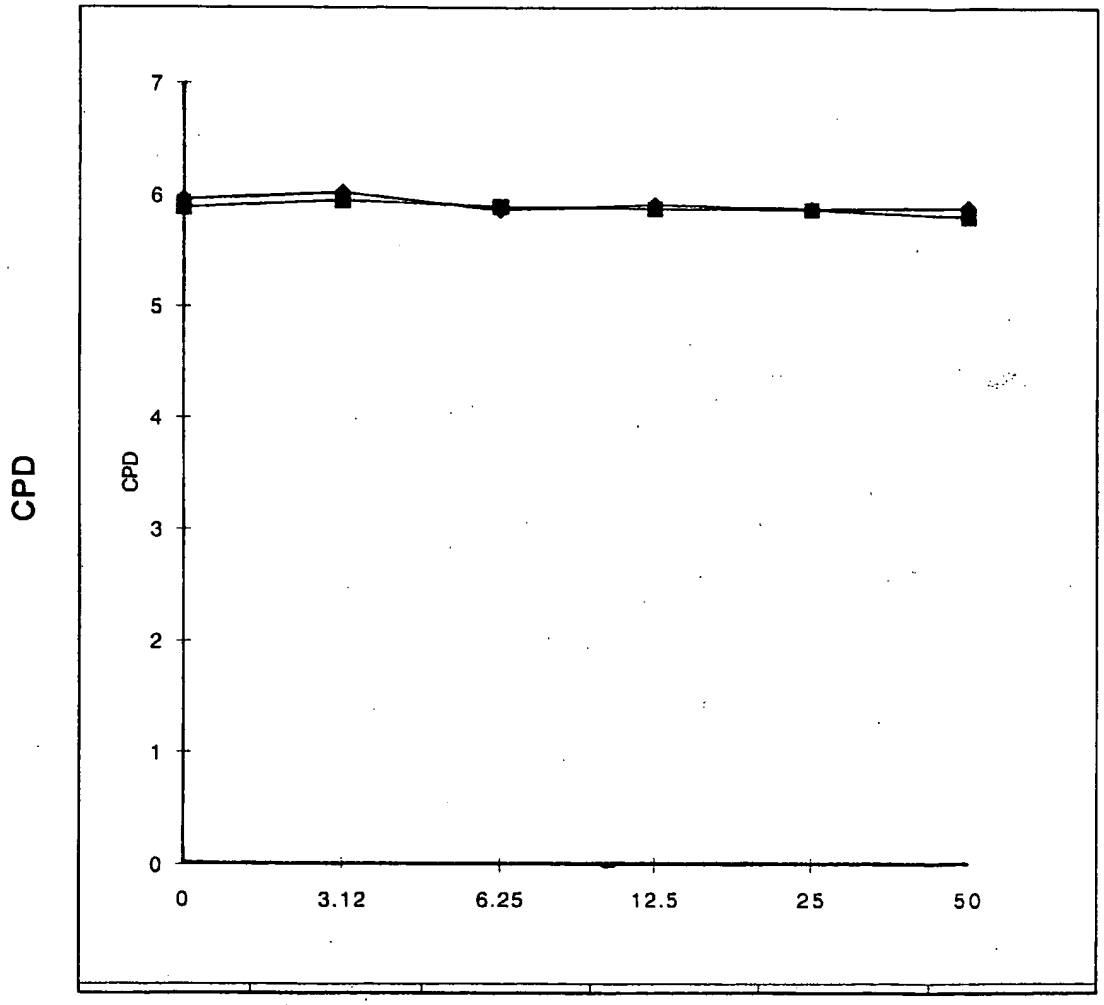


LEGEND:

1. MW
2. ALVA 41: 40 ug
3. ALVA 41: 20 ug
4. DU 145: 40 ug
5. DU 145: 20 ug
6. HUMAN FIBROBLAST: 40 ug
7. HUMAN FIBROBLAST: 20 ug
8. LNCaP: 40 ug
9. LNCaP: 20 ug
10. MDCK1: 20 ug
11. MDCK1: 10 ug
12. PC3: 40 ug

FIGURE 147

EFFECT OF HUMAN PLASMA IgA ON DU145 CELL GROWTH WITH AND WITHOUT DHT



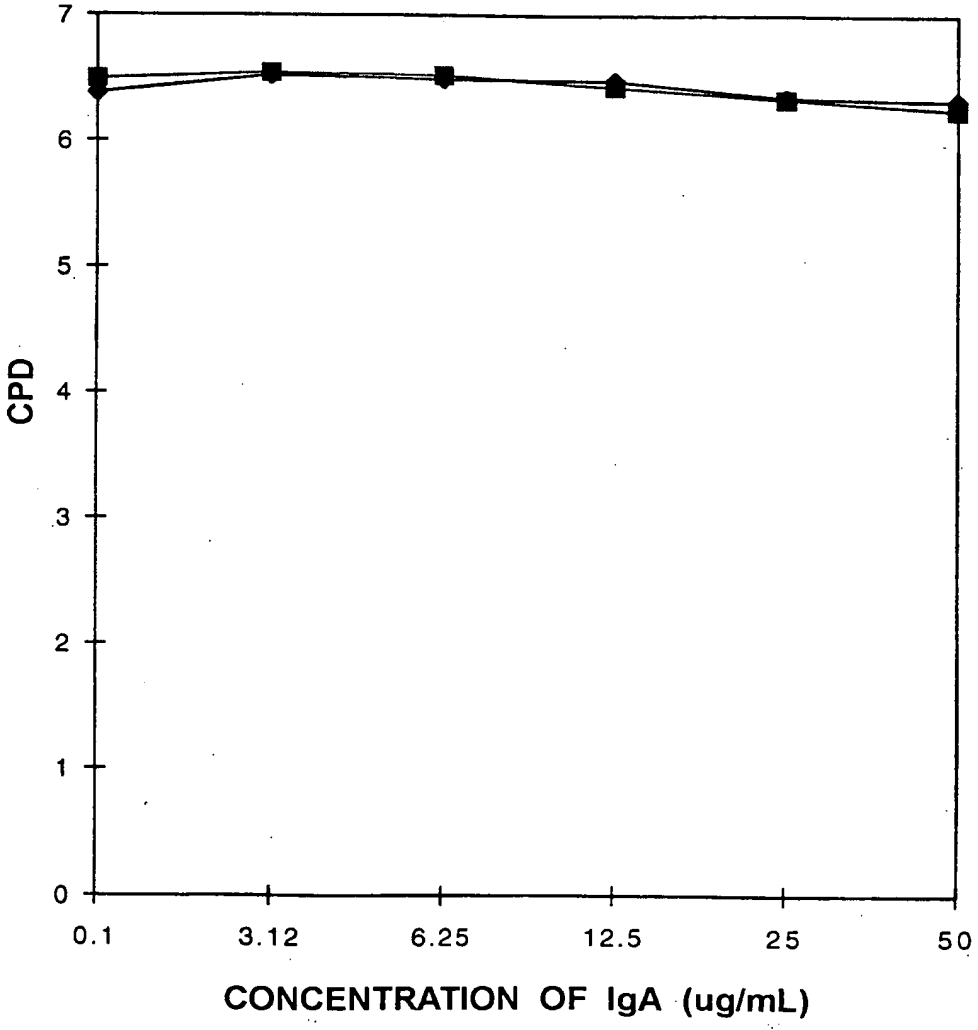
LEGEND:

—◆— = + DHT

—■— = – DHT

FIGURE 148

EFFECT OF HUMAN PLASMA IgA ON PC3 CELL GROWTH WITH AND WITHOUT DHT



LEGEND:

—◆— = + DHT

—■— = − DHT